

Synthesis and metabolism of 2,2,17,17-tetramethylstearic acid. I¹

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With 11 figures in the text

Recently we have found that after feeding 2,2-dimethyl[1-¹⁴C]stearic acid to rats 90 per cent of the absorbed activity could be recovered in the urine mainly as 2,2-dimethyladipic acid (Bergström, Borgström, Tryding and Westöö, 1). No isotope was found in the expired carbon dioxide, indicating that the β -oxidation from the carboxyl end of the molecule was blocked. 2,2-Dimethylnonadecanoic acid has been found to behave similarly, 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid being formed (Tryding and Westöö, 2). In contrast, 2-monomethylstearic acid is metabolized in a way similar to that of the unbranched stearic acid (3).

We have now synthesized a long chain fatty acid which should not be metabolized either by β -oxidation from the carboxyl end or by ω -oxidation followed by β -oxidation from the ω -end, viz. 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid, and we have studied the metabolism of this acid in rats.

Experimental

Syntheses

2,2,17,17-Tetramethyl[1-¹⁴C]octadecanoic acid.—15,15-Dimethylhexadecanoic acid (1.8 g of crude product, prepared by electrolysis of 3,3-dimethylbutyric acid and 13-methoxycarbonyltridecanoic acid, Tryding and Westöö, to be published), 3-methoxy[¹⁴C]carbonyl-3-methylbutyric acid (0.9 g of crude product) and sodium (0.028 g) were dissolved in methanol (30 ml) and electrolysed between platinum electrodes (current density 0.3 amp/cm²) until pH 7 (cf. Stållberg-Stenhagen, 4). The precipitate formed, mainly 2,2,29,29-tetramethyltriacontane, was filtered off at room temperature, boiled with methanol and after cooling to room temperature again removed by filtration. The methanol was distilled off from the combined methanol solutions, the dry residue was extracted with ether and the ether solution was evaporated to dryness. The product obtained, 0.85 g, was chromatographed on alumina (40 g of an alumina which had been washed with hydrochloric acid and water and dried at 180° overnight). The petroleum fractions were discarded, the benzene fractions collected and after removal of the benzene hydrolysed with 4 g

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of potassium hydroxide, 25 ml of ethanol and 1 ml of water in an autoclave at 120° during 4 hours. Water (20 ml) was added and the solution was extracted with light petroleum. The petroleum solution, which contained the 2,2,17,17-tetramethyloctadecanoic acid, was extracted with 50 per cent ethanol, the alcohol solution was acidified and extracted with ether. Evaporation of the ether solution gave 0.34 g of crude product. Recrystallization from methanol and petroleum ether resulted in colourless crystals (0.17 g), m.p. 46.6–46.8°. Found: C 77.5; H 12.9; equiv. wt. 341. $C_{22}H_{44}O_2$ requires C 77.6; H 13.0; equiv. wt. 341.

2,2,17,17-Tetramethyloctadecanedioic acid.—A solution of 3-methoxycarbonyl-3-methylbutyric acid (7.7 g), ethyl hydrogen suberate (14.3 g) and sodium (0.30 g) in methanol (120 ml) was electrolysed between Pt-electrodes until pH = 7 (cf. Stållberg-Stenhagen, 4). The methanol was evaporated and the residue was extracted with ether saturated with water. The half esters were removed on a column of Amberlite IRA-400.

After evaporation of the ether, the esters were partially hydrolysed with a solution of potassium hydroxide (8 g) in water (20 ml) and ethanol (80 ml) at room temperature for 16 hours. Water (50 ml) was added, and the solution was extracted with light petroleum to remove neutral esters. The ethanol–water layer was then acidified and again extracted with light petroleum. The petroleum solution was washed with water, dried with sodium sulphate and evaporated to dryness.

The half esters obtained were chromatographed on silica gel in methylene chloride solution. The main band, 9-methoxycarbonyl-9-methyldecanoic acid (2.0 g), and sodium (0.02 g) were dissolved in methanol and electrolysed between platinum electrodes until pH = 7. The solvent was evaporated, the residue was dissolved in ether saturated with water and passed through a column with Amberlite IRA-400. After evaporation of the ether, the ester was hydrolysed with a solution of potassium hydroxide (3 g) in water (2 ml) and ethanol (18 ml) in an autoclave at 120° for four hours. Water (15 ml) was added to the solution, which was then extracted with light petroleum to remove hydrocarbons. The ethanol–water layer was acidified and extracted with ether. The ether solution was washed with water, dried with sodium sulphate and evaporated to dryness. The acid obtained was purified by crystallization from ethanol and from benzene–light petroleum. M.p. 96.5°. Found: C 71.0; H 11.4. $C_{22}H_{42}O_4$ requires C 71.3; H 11.4.

Chromatography

The reversed-phase partition chromatographic technique (Howard and Martin, 5) used in this investigation was essentially the same as described earlier (1, 2). However a few new systems originally introduced by Bergström and Sjövall (6) for the separation of bile acids have been added. The chromatographic systems of this investigation are summarized in Table 1. The supporting medium was hydrophobic kieselguhr (Hyflo supercel treated with dimethyldichlorosilane) for all the solvent systems. Generally 4.5 g columns with 4 ml of the stationary phases were prepared, but also 9 g columns were used. The fractions (2 ml) collected by a time-operated fraction collector, were titrated with 0.02 *N* methanolic sodium hydroxide solution.

Metabolic experiments

The 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid was crystallized repeatedly and subjected to partition chromatography with phase system A to ensure that no traces

Table 1. Chromatographic systems

Supporting medium: Hydrophobic kieselguhr (Hyflo supercel treated with dimethyldichlorosilane).

System	According to	Stationary phase	Moving phase
A	Howard and Martin (5)	Paraffin oil (25 ml)	Acetone : water 3 : 1, v/v (225 ml : 75 ml)
B	Bergström and Sjövall (6) Bergström, Borgström Tryding and Westöö (1)	2-ethylhexanol: chloroform 1 : 1, v/v (25 ml : 25 ml)	methanol : water 2 : 3, v/v (120 ml : 180 ml)
E	Bergström and Sjövall (6) Sjövall (7)	heptane : chloroform 1 : 9, v/v	methanol : water 3 : 2, v/v (180 ml : 120 ml)
F	Modified from Sjövall (7)	heptane : chloroform 1 : 4, v/v	methanol : water (205 ml : 95 ml)

of ^{14}C -labelled contaminants remained. An olive oil solution containing 2 per cent of 2,2,17,17-tetramethyl[1- ^{14}C]stearic acid (s.a.: $0.2 \mu\text{C}/\text{mg}$) was prepared. The radioactivity was generally determined with a gas flow counter (background effect: 30 cpm) but in a few instances a mica window GM-counter (background effect: 25 cpm) was used. With these counters the specific activity of the 2,2,17,17-tetramethylstearic acid used in the above mentioned stock solution was found to be 150,000 cpm/mg and 25,000 cpm/mg respectively. As a rule the samples were plated directly on aluminium planchets and counted in infinitely thin layers. When correction for self-absorption was necessary it was performed as described earlier (1). The rats, which were from the same stock as those of our earlier investigations, were all of male sex. Generally 1 ml of the stock solution was administered by gastric intubation under a light ether anaesthesia. For the special techniques used, i.e., for cannulating the lymph or bile ducts, see below under Results.

Results

Administration of 2,2,17,17-tetramethylstearic acid to rats with a lymph fistula.—One ml of the olive oil solution containing 2 per cent of 2,2,17,17-tetramethyl[1- ^{14}C]stearic acid was fed to two rats provided with a lymph fistula. The procedure for the cannulation of the thoracic duct and the pre- and postoperative treatment of the animals were carried out according to Bergström, Borgström and Blomstrand (8). The lymph produced during 24 hours after the administration of the labelled acid was collected into ethanol. In addition the feces were collected during three days. The lymph lipids were extracted and fractionated with standard methods of this institute (8, 9). The results are summarized in Table 2. When a neutral fat fraction was hydrolysed and the acids obtained were chromatographed with phase system A, the activity was found to coincide with the titration peak of added unlabelled tetramethylstearic acid.

Isotope recovery in the expired carbon dioxide after the administration of carboxyl-labelled 2,2,17,17-tetramethylstearic acid to rats.—A male 250 g rat was given through

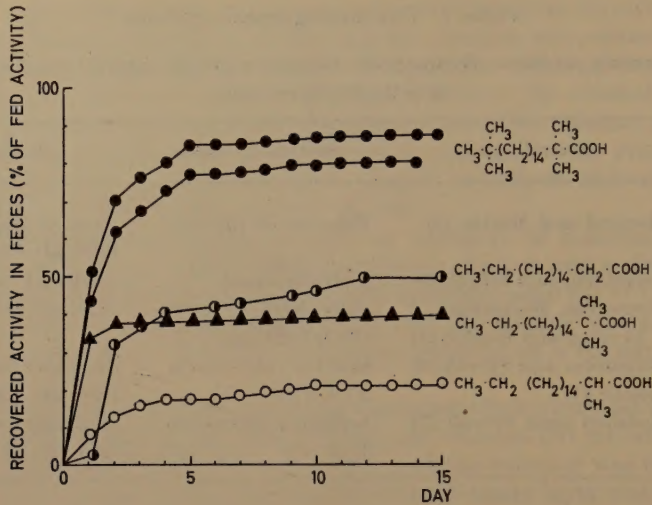


Fig. 1. Accumulation curves for the radioactivity excreted in the feces from rats fed 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid, 2,2-dimethyl[1-¹⁴C]stearic acid, 2-methyl[1-¹⁴C]stearic acid and [1-¹⁴C]stearic acid.

a stomach tube 1 ml of an olive oil solution containing 2 per cent of free 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid. According to Lee (10) this corresponds to 0.3 ml per sq.dm of body surface. The expired carbon dioxide was collected and the isotope content was determined as described earlier (1). With the method used, no significant activity was detected in the CO₂ specimens, which were taken during two days.

Isotope recovery in the feces after feeding 2,2,17,17-tetramethylstearic acid, 2,2-dimethylstearic acid, 2-methylstearic acid and stearic acid to rats.—Two different rats were given by stomach tube 1 ml of the olive oil solution containing 2 per cent of 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid and the feces were collected daily during a fortnight after the administration of the acid. In parallel experiments [1-¹⁴C]stearic acid (s.a.: 130,000 cpm/mg), 2-methyl[1-¹⁴C]stearic acid (s.a.: 110,000 cpm/mg, cf. ref. 3) and 2,2-dimethyl[1-¹⁴C]stearic acid (s.a.: 250,000 cpm/mg, cf. ref. 1) were fed to rats in a 2 per cent solution in olive oil.

Table 2. Recovery of radioactivity in the feces and the lymph after feeding 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid to lymph fistula rats.

Per cent of fed activity recovered in the feces (3 days)	Per cent of fed activity recovered in the lymph (24 hours)	Per cent of lymph fat activity found as		
		neutral fat	unesterified fatty acids	phospholipids
38.0	40.8	82.7	14.2	3.1
35.2	36.1	87.0 ^a	10.1 ^a	2.9

^a The specific activities of the neutral fat fatty acids and the unesterified fatty acids were 2227 cpm/mg and 3508 cpm/mg, respectively.

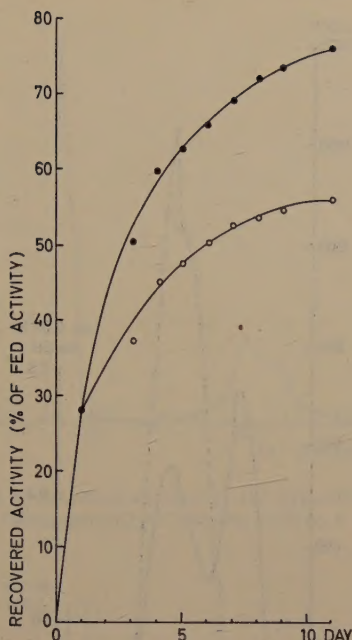


Fig. 2. Accumulation curves for the radioactivity recovered in the feces (unfilled circles) and in the bile plus the feces (filled circles).

After homogenization the feces were refluxed overnight with 5 *N* ethanolic potassium hydroxide solution. The alcohol was evaporated and the acidified mixtures were extracted with peroxide-free ether. The accumulation curves of the excreted isotopes are shown in Fig. 1.

Isotope recovery in the bile after the administration of 2,2,17,17-tetramethylstearic acid to rats.—A rat was fed 1 ml of the stock solution containing ^{14}C -labelled 2,2,17,17-tetramethylstearic acid. Twenty-four hours after the administration the rat was provided with a bile fistula (cf. Borgström, Sjövall and Voltz, 11). The bile and the feces were collected daily. The bile was collected in ethanol, and the activity was determined by direct plating of a suitable aliquot after filtration. The feces were digested with potassium hydroxide before acidification and extraction with ether as described above. The accumulation curves for the activities recovered in the feces and in the bile plus the feces are shown in Fig. 2.

Another rat was fed 0.7 ml of an olive oil solution containing 2,2,17,17-tetramethyl- $[1-^{14}\text{C}]$ stearic acid (2,520,000 cpm). Twelve hours after the feeding when the intestinal absorption was nearly complete, as judged by the lymph fistula experiments, the bile duct was cannulated. The bile excreted during 24 hours after the establishment of the fistula contained 330,000 cpm, i.e., 13.1 per cent of the fed activity. During the next 5 days 14.8, 5.4, 3.8, 1.0, and 0.2 per cent respectively of the fed amount were detected in the bile specimens. Thus 38.3 per cent of the fed isotope were found to be excreted via the bile in six days.

Isotope recovery in the bile after the administration of $[1-^{14}\text{C}]$ palmitic acid to a rat.—A rat was fed 1 ml of a 2 per cent olive oil solution of $[1-^{14}\text{C}]$ palmitic acid (s.a.:

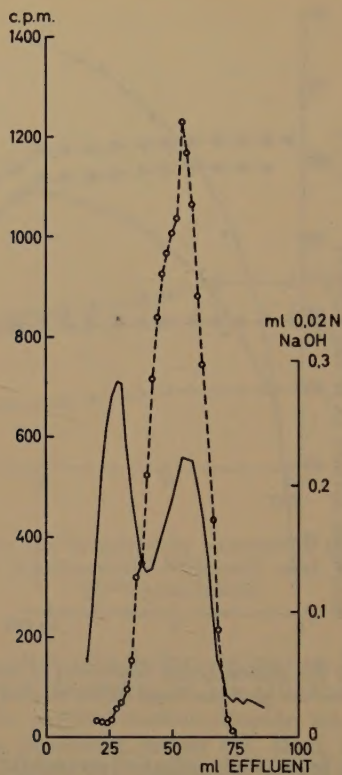


Fig. 3. Identification of unchanged palmitic acid in the bile from a rat fed $[1-^{14}\text{C}]$ palmitic acid. Moving phase: acetone: water 65:35 (v/v). Stationary phase: paraffin oil. Solid line: alkaline consumption. Broken line: radioactivity.

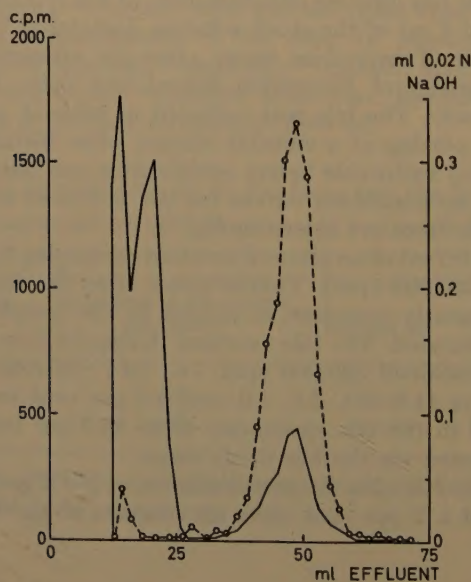


Fig. 4. Identification of unchanged 2,2,17,17-tetramethylstearic acid in an ether extract of feces. Phase system A. Solid line: alkaline consumption. Broken line: radioactivity.

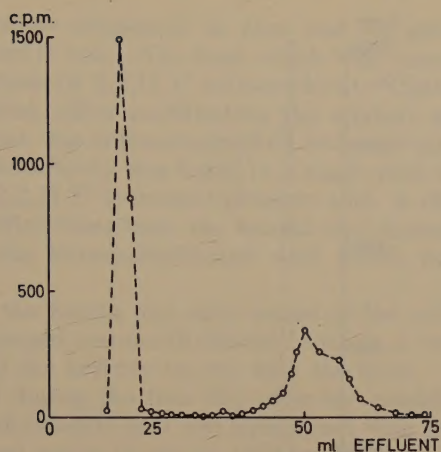


Fig. 5. Partition chromatogram of an ether extract of the saponified feces from the second day after feeding 2,2,17,17-tetramethyl[1- 14 C]stearic acid to a rat. Phase system A.

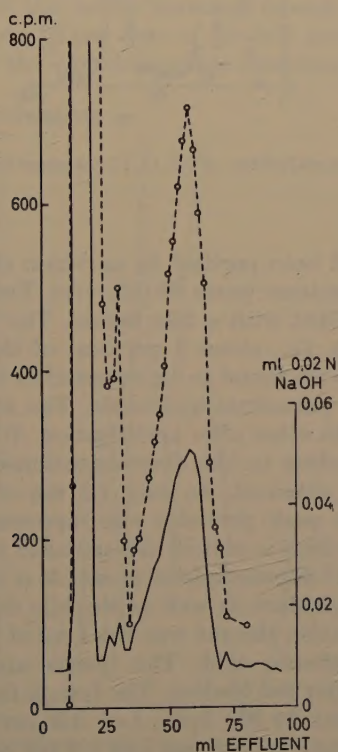


Fig. 6. Identification of 2,2,17,17-tetramethyloctadecanedioic acid in an ether extract of hydrolysed bile of a rat fed 2,2,17,17-tetramethyl[1- 14 C]stearic acid. Phase system F. Solid line: alkaline consumption. Broken line: radioactivity.

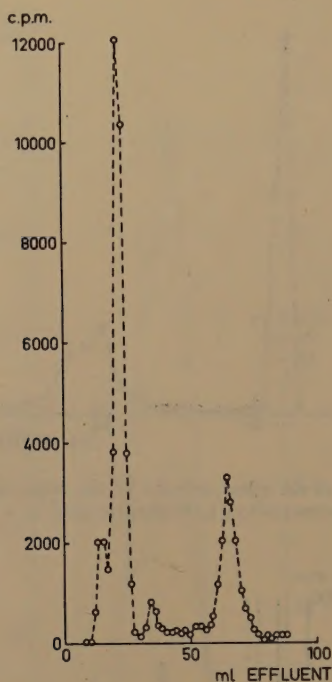


Fig. 7. Chromatography of bile metabolites of 2,2,17,17-tetramethylstearic acid. Phase system E.

475,000 cpm/mg), which had been purified by partition chromatography with phase system A (moving phase: acetone:water 65:35, v/v). Twelve hours after the administration the rat was provided with a bile fistula. The bile from the following 24 hours contained 86,500 cpm, i.e., about 1 per cent of the fed activity. During the next two days 17,500 cpm were found to be excreted in the bile. This last specimen was saponified in ethanolic potassium hydroxide. The alcohol was evaporated and the acids were extracted with ether after acidification. When the extracted material was chromatographed according to the above-mentioned system A the chromatogram shown in Fig. 3 was obtained. As only 1.2 mg of carrier palmitic acid was added, the second titration peak probably also represents naturally occurring palmitic acid (and oleic acid which is eluted like palmitic acid in this system).

Administration of 2,2,17,17-tetramethylstearic acid to a rat with both a lymph and a bile fistula.—The main lymph duct as well as the bile duct were cannulated in one rat. The day after the operation the rat was fed 1 ml of the fat mixture containing 2,2,17,17-tetramethyl[1- 14 C]stearic acid. The lymph and the bile were collected during the next two days after the feeding. The lymph from the first six hours after the administration contained 80,700 cpm, i.e., 3.3 per cent of the fed activity. During the next 6 and 12 hours 1.2 per cent and 1.3 per cent respectively were found in addition. In two days a total of 6.2 per cent of the fed isotope had been recovered in the lymph. During the same period the bile activity was 22,000 cpm, i.e., 0.9 per cent of the fed activity or 15 per cent of the lymph activity.

Isolation of the isotopic compounds in feces and bile after feeding 2,2,17,17-tetramethyl[1- 14 C]stearic acid to rats.—The feces which were excreted during the first day after the administration of 2,2,17,17-tetramethyl[1- 14 C]stearic acid to a rat were collected and saponified. After acidification the mixture was extracted with ether and part of the extract was chromatographed with solvent system A. As shown in Fig. 4 almost all of the activity was found in a single peak appearing at the position of added unlabelled 2,2,17,17-tetramethylstearic acid. A chromatogram of an ether extract of the saponified feces from the second day showed a distinct front peak, except for that of the tetramethylstearic acid which was now less pronounced (Fig. 5).

In the feces from the fourth day only traces of the activity were found at the position of the unchanged tetramethylstearic acid in a chromatogram with phase system A. The rest of the activity moved with the front.

The bile recovered during the first day after the establishment of a bile fistula on a rat fed tetramethylstearic acid was hydrolysed with potassium hydroxide (8 g) in ethanol (50 ml) and water (4 ml) at 120° for 6 hours. After evaporation of the alcohol the acids were extracted with ether from the acidified water phase. Part of this extract containing 75,000 cpm was chromatographed together with synthetic 2,2,17,17-tetramethyloctadecanedioic acid (7.5 mg) on a 4.5 g column with solvent system F (Fig. 6). Most of the active material moved with the front, but part of the activity (7,400 cpm, i.e., 10 per cent of the bile activity) was found to coincide with the titration peak of the added, inactive dicarboxylic acid. The material from

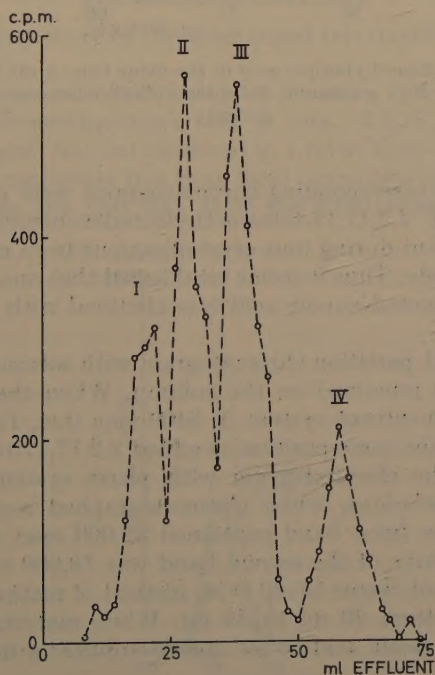


Fig. 8. Chromatography of urinary metabolites of 2,2,17,17-tetramethylstearic acid. Phase system B.

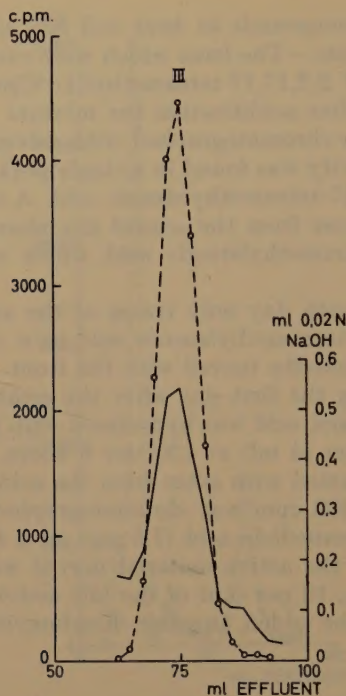


Fig. 9. Identification of 2,2-dimethyladipic acid in the urine from a rat fed 2,2,17,17-tetramethylstearic acid. Phase system B. 9 g column. Solid line: alkaline consumption. Broken line: radioactivity.

this peak and from two corresponding chromatograms were pooled and crystallized together with inactive 2,2,17,17-tetramethyloctadecanedioic acid. The specific activity remained constant during four crystallizations from methanol and benzene-light petroleum alternately. Thus it seems established that one of the bile metabolites of 2,2,17,17-tetramethyloctadecanoic acid was identical with 2,2,17,17-tetramethyloctadecanedioic acid.

In the last mentioned partition chromatogram with solvent system F 11,000 cpm of the isotopic material remained on the column. When the methanol eluate was rechromatographed with solvent system A, 9400 cpm (i.e., 12.5 per cent of the bile activity) were found at the same position as added 2,2,17,17-tetramethylstearic acid.

The front peak of the chromatogram with phase system F was found to contain two different metabolites, when chromatographed according to a modified system (E) (Fig. 7). The front band contained 32,000 cpm (43 per cent of the bile activity), while the activity of the second band was 16,000 cpm. When the column was eluted with methanol:water 55:40 (v/v) instead of methanol:water 60:40 (v/v) the front peak moved from 20 ml to 35 ml. When material from this peak was oxidized with Cr_2O_3 in acetic acid at 50° for 45 minutes a more polar product was obtained.

As judged from chromatograms the active products found in the bile were also present in the feces. The feces from the first day after the establishment of a bile

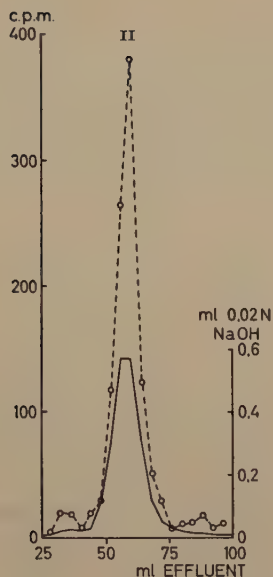


Fig. 10. Identification of 2,2-dimethylglutaric acid (cf. Fig. 9).

fistula contained distinctly more of the unchanged tetramethylstearic acid than did the corresponding bile.

Isotope recovery in urine and identification of the main isotopic urinary compounds after feeding 2,2,17,17-tetramethylstearic acid to rats.—2,2,17,17-Tetramethyl[1- ^{14}C]-stearic acid (1,700,000 cpm) was fed dissolved in 1 ml of olive oil to a rat, from which the urine was collected completely free from fecal impurities (cf. Tryding and Westöö, 2). When the urine from the first day after the administration was acidified and

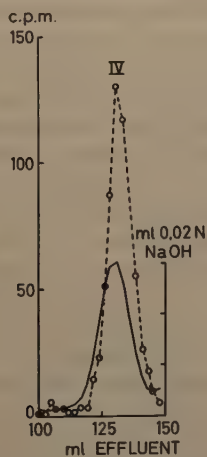


Fig. 11. Identification of 2,2-dimethylpimelic acid (cf. Fig. 9).

When the continuous ether extract of the urine, earlier extracted with ether three times, was chromatographed according to system B almost all the activity appeared in the front band. This was found to be heterogeneous, as it was distinctly reduced after hydrolysis, when part of the activity appeared at the positions of the other active bands. As all the active material could not be hydrolysed there must also be nonconjugated products, which are more polar than the isolated metabolites. The mean figures obtained from the chromatographic analysis of the urines from three rats are seen in Fig. 12.

Discussion

2,2,17,17-Tetramethyl[1-¹⁴C]stearic acid was found to be well absorbed by rats, mainly via the lymph (cf. Table 2) but presumably also via the portal vessels as a significant amount of the absorbed activity was excreted in the bile after feeding the acid to a rat provided with both a lymph and a bile fistula. This is in agreement with earlier results obtained on the absorption of 2,2-dimethylstearic acid (12) and 2,2-dimethylnonadecanoic acid (2). However, the possibilities of lymph vessels by-passing the fistula and other factors of error pointed out by Bergström, Blomstrand and Borgström (8) must be kept in mind.

Most of the lymph activity (83 to 87 per cent) was found in esters of the unchanged tetramethylstearic acid. A considerable amount (10 to 14 per cent) was associated with the unesterified fatty acids, while just a few per cent of the activity were found in the phospholipids. In one of the experiments the specific activity of the free fatty acids was 158 per cent of the specific activity of the neutral fat fatty acids. These findings are in good accordance with the results of earlier investigations on the distribution of long chain, branched fatty acids in the lymph (2, 3, 9, 12). In the absence of bile from the small intestine only a small percentage (6.2 per cent) of the fed 2,2,17,17-tetramethylstearic acid appeared in the lymph. The poor intestinal absorption of long chain fatty acids in the absence of bile was demonstrated by Siperstein, Chaikoff and Reinhardt (13) and Borgström (14).

After feeding 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid no significant activity was found in the expired carbon dioxide in two days. The same result was obtained after feeding 2,2-dimethyl[1-¹⁴C]stearic acid to rat (1). In identical experiments with [1-¹⁴C]stearic acid and 2-methyl[1-¹⁴C]stearic acid about 25 per cent of the absorbed activity were excreted as carbon dioxide in two days (Tryding and Westöö, 3).

Because the carbon atom 17 is quaternary no dehydrogenation can take place between the 16 and 17 carbon atoms, and the molecule cannot be β -oxidized from the ω -end in the usual way (cf. 1, 2). In spite of this urine from four different rats fed 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid was found to contain 3.6 per cent (in mean) of the fed isotope. The main metabolites were identified as 2,2-dimethyladipic acid, but also 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid were found to be excreted (cf. Fig. 12). This must be explained by the existence of other degradation mechanisms of this long chain fatty acid than the ordinary β -oxidation or ω -oxidation followed by β -oxidation. Most probably the carbon chain is split between the methyl branchings, the cleavages occurring both at odd and even carbon atoms. Schoenheimer and Rittenberg (15) have demonstrated the existence in the body of enzymes capable of introducing double bonds in the middle of the fatty acid molecule. Lang and Adickes (16) have shown that the liver contains an enzyme that can desaturate the stearic acid chain in the middle giving rise to oleic acid. However

Bernhard and Gloor (17) have found that oleic acid is not oxidized to azelaic acid *in vivo*. This was in contrast to 9,10-dihydroxystearic acid and 9,10-diketostearic acid which were both found to produce azelaic acid in the body.

The finding that 2,2-dimethyladipic acid was the main metabolite excreted in the urine of rats fed 2,2,17,17-tetramethylstearic acid might be explained by a degradation of the acid from the ω -end. If two of the methyl groups at the ω -end are oxidized and if one of the carboxyl groups formed splits off carbon dioxide, the resulting monomethyl branched end can easily be β -oxidized (3). An analogous process with oxidation of an α -methyl group should give rise to radioactive carbon dioxide. However no significant activity has been found in the expired air, but with the methods used 3 per cent or less of the fed activity would not have been detected.

During a period of two weeks 80 to 85 per cent of the active metabolites of 2,2,17,17-tetramethyl[1-¹⁴C]stearic acid were excreted in the feces. For comparison [1-¹⁴C]-stearic acid, 2-methyl[1-¹⁴C]stearic acid and 2,2-dimethyl[1-¹⁴C]stearic acid were administered in parallel experiments. In 15 days the isotope recovery in the feces from rats fed these acids did not exceed 50 per cent of the fed amount (cf. Fig. 1).

When a bile fistula was made one day after feeding 2,2,17,17-tetramethyl[1-¹⁴C]-stearic acid to a rat, about equal amounts of the radioactivity were found in the bile and in the feces. Part of the bile activity was identified as the unaltered acid, but also the corresponding dicarboxylic acid was isolated. In addition more polar active bile compounds were found. They have been separated by partition chromatography and presumably have the same carbon chain as 2,2,17,17-tetramethyloctadecanoic acid but contain hydroxy groups in the side chains. These products are still being investigated. The radioactive compounds identified in the bile were also present in the feces of this bile fistula rat. This indicates that the metabolites of tetramethylstearic acid are retransported to the intestinal contents also by other ways than via the bile, presumably via other digestive juices or via desquamation of the mucosa epithelium.

We have found that after feeding 2,2,17,17-tetramethylstearic acid to rats, 33 per cent of the fed activity could be recovered in the bile in three days. In parallel experiments with [1-¹⁴C]palmitic acid only about one per cent of the acid was found in the bile during the same period. The excretion of endogenous fatty acids does not seem to be of any great importance under normal conditions, when the fecal fatty acids probably mainly originate from the diet. Theories have been proposed, that great amounts of fat are normally excreted into the intestinal tract (cf. 18, 19). The fat should be reabsorbed from the intestine in the presence of bile. The feces from bile fistula animals contain increased amounts of fatty acid (cf. 18). However, according to Holasek (20) these mainly come from the intestinal microorganism. A discussion on the origin of fecal fatty acids was recently published by Bergström and Blomstrand (21), who have studied the intestinal absorption of fat in man. For a review see also (22).

SUMMARY

1. 2,2,17,17-Tetramethyl[1-¹⁴C]octadecanoic acid and 2,2,17,17-tetramethyloctadecanedioic acid have been synthesized.

2. When the former was administered in an olive oil solution to adult rats it was found to be well absorbed via the lymphatics, like the ordinary, long chain fatty acids. The tetramethylstearic acid was recovered in the unchanged form in the lymph, mainly incorporated into esters.

3. No significant activity was found in the expired carbon dioxide with the method used.
4. Almost all (up to 85 per cent) of the fed isotope could be recovered from the feces during one week. A retransport of the absorbed, labelled material to the intestinal lumen takes place via the bile but also via other ways.
5. About 13 per cent of the isotope in the bile was present in unchanged tetramethylstearic acid. Another isotopic compound was 2,2,17,17-tetramethyloctadecanedioic acid which represented about 10 per cent of the total bile activity. The two main bile metabolites were both more polar than the dicarboxylic acid.
6. About 3.5 per cent of the fed activity was recovered in the urine. The main product excreted was identified as 2,2-dimethyladipic acid but also 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid were found indicating that the tetramethylstearic acid is degraded by a mechanism different from the ordinary β - and ω -oxidations.

We are greatly indebted to Professor Sune Bergström who suggested this investigation and who placed the labelled stearic and palmitic acids at our disposal.

The valuable technical assistance of Miss Kerstin Sjöström is gratefully acknowledged.

This investigation was supported by grants from *Statens Naturvetenskapliga Forskningsråd*, from *Knut och Alice Wallenbergs Stiftelse* and from the *Medical Faculty of the University of Lund*.

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REFERENCES

1. BERGSTRÖM, S., BORGSTRÖM, B., TRYDING, N., and WESTÖÖ, G., *Biochem. J.* **53**, 604 (1954).
2. TRYDING, N., and WESTÖÖ, G., *Acta Chem. Scand.* **10**, 1234 (1956).
3. TRYDING, N., and WESTÖÖ, G., *Acta Chem. Scand.* **11**, 427 (1957).
4. STÄLLBERG-STENHAGEN, S., *Arkiv Kemi* **2**, 95 (1950).
5. HOWARD, G. A., and MARTIN, A. J. P., *Biochem. J.* **46**, 532 (1950).
6. BERGSTRÖM, S., and SJÖVALL, J., *Acta Chem. Scand.* **5**, 1267 (1951).
7. SJÖVALL, J., *Acta Physiol. Scand.* **29**, 232 (1953).
8. BERGSTRÖM, S., BLOMSTRAND, R., and BORGSTRÖM, B., *Biochem. J.* **53**, 600 (1954).
9. BORGSTRÖM, B., and TRYDING, N., *Acta Physiol. Scand.* **37**, 127 (1956).
10. LEE, M. O., *Am. J. Physiol.* **89**, 24 (1929).
11. BERGSTRÖM, S., SJÖVALL, J., and VOLTZ, J., *Acta Physiol. Scand.* **30**, 22 (1953).
12. BLOMSTRAND, R., TRYDING, N., and WESTÖÖ, G., *Acta Physiol. Scand.* **37**, 91 (1956).
13. SIPERSTEIN, M. D., CHAIKOFF, I. L., and REINHARDT, W. O., *J. Biol. Chem.* **198**, 111 (1952).
14. BORGSTRÖM, B., *Acta Physiol. Scand.* **28**, 279 (1953).
15. SCHOENHEIMER, R., and RITTENBERG, D., *J. Biol. Chem.* **113**, 505 (1936).
16. LANG, K., and ADICKES, F., *Hoppe-Seyler's Z. physiol. Chem.* **262**, 123 (1939).
17. BERNHARD, K., and GLOOR, U., *Helv. Chim. Acta* **35**, 608 (1952).
18. BERNHARD, K., RITZEL, G., and HUG, E., *Helv. Physiol. et Pharmacol. Acta* **10**, 68 (1952).
19. BERNHARD, K., and RITZEL, G., *Helv. Physiol. et Pharmacol. Acta* **11**, 166 (1953).
20. HOLASEK, A., *Hoppe-Seyler's Z. physiol. Chem.* **298**, 224 (1954).
21. BERGSTRÖM, S., and BLOMSTRAND, R., in *Biochemical Problems of Lipids* (G. POPJÁK and E. LE BRETON, eds.), Butterworth Scientific Publications, London (1956).
22. BERGSTRÖM, S., and BORGSTRÖM, B., *Ann. Rev. Biochem.* **25**, 177 (1956).

Tryckt den 4 maj 1957

Uppsala 1957. Almqvist & Wiksells Boktryckeri AB

Intestinal absorption and metabolism of 2,2-dimethylstearic acid in man

By NILS TRYDING

With 4 figures in the text

2,2-Dimethylstearic acid was well absorbed when fed to rats in an olive oil solution (Bergström, Borgström, Tryding and Westöö, 1). The main metabolic end product was 2,2-dimethyladipic acid, which was rapidly excreted in the urine. As there was no hydrogen at the α -carbon atom, the usual β -oxidation from the carboxyl end was hindered and the molecule was instead oxidized at the ω -end. Presumably the resulting dicarboxylic acid then underwent six β -oxidations from the unsubstituted end.

In the present work the acid has been given to two human subjects. In the first part of the study 1 g of the unlabelled 2,2-dimethylstearic acid was ingested by a healthy male student. The urine was collected and 2,2-dimethyladipic acid was isolated with the technique earlier described (1). In the second part a few mg of 2,2-dimethyl[1- 14 C]stearic acid were given to a male patient with a chylothorax due to a malignant tumor. In addition to the urine analyses also analyses of lymph specimens and feces were performed.

Experimental

The acids used in this investigation were those described in an earlier paper from this institute (1). The specific activity of the 2,2-dimethyl[1- 14 C]stearic acid was 0.19 μ C/mg. The determinations of radioactivity were performed in a "Tracerlab" gas-flow counter (1 μ C = $\sim 8.3 \times 10^5$ cpm, background effect: 30 cpm). The samples were plated directly on aluminium planchets.

The chromatographic separations were performed as described earlier (1, 2). The following solvent systems were used:

Stationary phase	Moving phase
B 2-ethylhexanol/chloroform, 1/1	methanol/water, 2/3
C butanol	water

Generally 9 g of the supporting medium (hydrophobic kieselguhr) and 8 ml of the stationary phases were used.

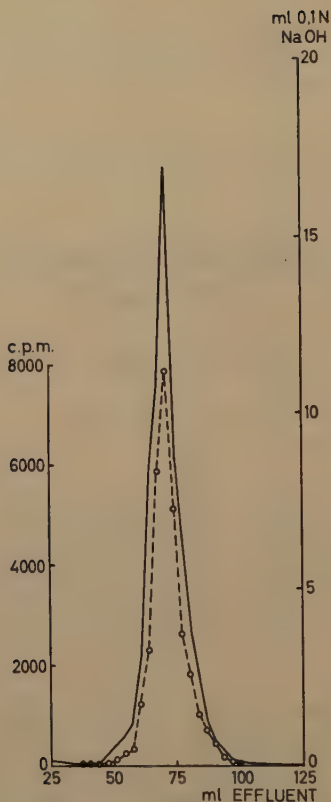


Fig. 1. Identification of 2,2-dimethyladipic acid. Phase system B. Solid line: alkaline consumption. Broken line: radioactivity.

Results

I.—1 g of 2,2-dimethylstearic acid was dissolved in olive oil and eaten on a piece of white bread by a 26-year-old male student. The urine from the first day was hydrolysed with KOH. After acidification with hydrochloric acid it was extracted three times with newly distilled ether. The ether was washed with small portions of water and evaporated. The residue (1.17 g) was chromatographed on a 45 g column with phase system B. The material of the titration peak, which appeared after elution with 300–450 ml of the moving phase, was rechromatographed on a 9 g column with the same phases.

The second-day-urine was also subjected to alkaline hydrolysis before acidification and extraction with ether. The residue of the ether extract weighed 1.35 g. It was chromatographed as described above together with 2,2-dimethyl[1- ^{14}C]adipic acid (5 mg), which had been isolated from the urine of a rat fed 2,2-dimethyl[1- ^{14}C]stearic acid.

The material of the single active peak (74 mg) was rechromatographed on a 4.5 g column with the same phase system (B). The substance from the most active frac-

Table 1. Distribution of activity in the lymph after feeding 2,2-dimethyl[1- 14 C]-stearic acid.

Fraction	Weight of fatty acids mg	Specific activity cpm/mg
Neutral fat	588	38
Phospholipids	70	21
Free fatty acids	48	88

tions (43 mg) was pooled together with the corresponding material (148 mg) from the chromatogram of the first day urine. Fig. 1 shows the final chromatogram. It is seen that the activity curve of the added labelled dimethyladipic acid coincides with the titration curve. The specific activity of the acids in the three most active fractions remained constant during four crystallizations from water and from benzene-light petroleum, alternately.

II.—A 74-year-old man with a chylothorax due to a malignant tumor (for clinical data see Blomstrand, 3) was fed 21.0 mg of 2,2-dimethyl[1- 14 C]stearic acid (4 μ C) in an olive oil solution (1.2 ml). The olive oil was given on a piece of white bread in the morning.

The next morning 70 ml of the chylothorax content were aspirated. The lymph specimen (cf. 3) was refluxed for one hour with 1.5 l of ethanol-ether, 3/1. The extracted fat (854 mg) was separated into a neutral fat-fatty acid fraction and a phospholipid fraction on a 10 g column of silicic acid according to Borgström (4). The neutral fat was freed from unesterified fatty acids on Amberlite-IRA 400 (5). The phospholipids and the neutral fat were saponified with alcoholic KOH, the fatty acids of each fraction were weighed and the specific activities determined. The results are summarized in Table 1.

When the free fatty acids of the lymph were chromatographed according to system B, all the activity remained in the stationary phase. This was also the case when the neutral fat fatty acids were subjected to partition chromatography with the same solvents.

The urine from the first day after the administration of the dimethylstearic acid was acidified with hydrochloric acid and extracted three times with two volumes of ether. The total ether extract contained 900,000 cpm, while another 490,000 cpm

Table 2. Activity recovered in the urine and in the feces after oral administration of 2,2-dimethyl[1- 14 C]stearic acid.

Day no.	Per cent of fed activity found in	
	feces	urine
1	1	43
2+3	6	17
4+5	1	3

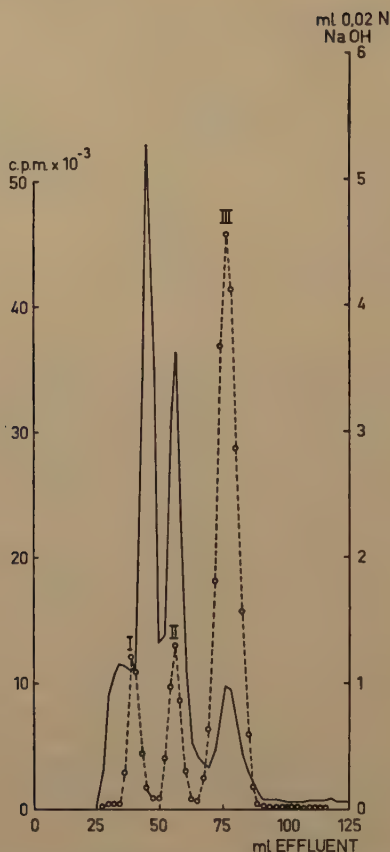


Fig. 2. Partition chromatography of an ether extract of urine from a man fed 2,2-dimethyl[1-¹⁴C]-stearic acid. Phase system B. Solid line: alkaline consumption. Broken line: radioactivity.

were extracted with butanol. In addition, 30,000 cpm could be extracted after alkaline hydrolysis of the water phase. The urine samples from the second plus third and fourth plus fifth days were found to contain 560,000 cpm and 100,000 cpm respectively (cf. also Table 2).

Part of the ether extracts of the first day urine was chromatographed together with inactive 2,2-dimethyladipic acid (6 mg) on a column with phase system B. As shown in Fig. 2, three distinct active peaks appeared. The third peak at 75 ml coincides with the titration peak of the added 2,2-dimethyladipic acid. The acids of the two most active fractions from active peak I with a weight of substance of 10.4 mg, were rechromatographed together with 14.7 mg of inactive 2,2-dimethylsuccinic acid on a column with phase system C. Fig. 3 shows the coincidence of the titration and activity curves. The identity of the active material with 2,2-dimethylsuccinic acid was confirmed by recrystallizations with added carrier. The specific activity was found to remain constant during five crystallizations from hydrochloric acid and from ethyl acetate alternately.

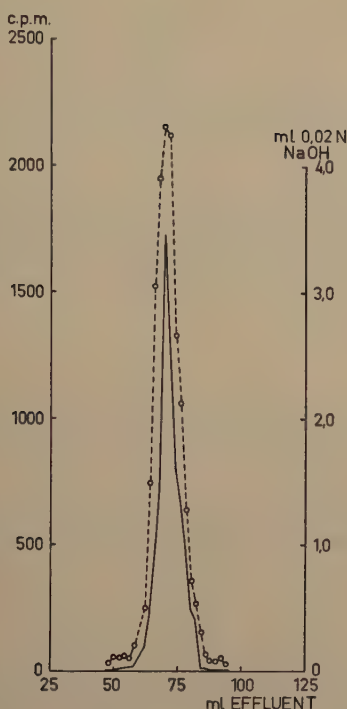


Fig. 3. Identification of 2,2-dimethylsuccinic acid. Phase system C. Solid line: alkaline consumption. Broken line: radioactivity.

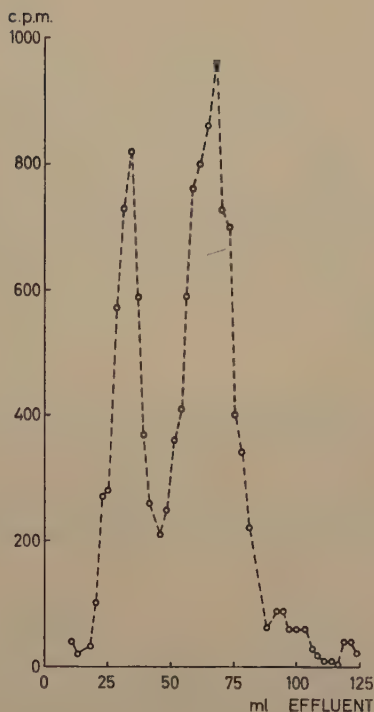


Fig. 4. Partition chromatography of a butanol extract of unhydrolysed urine from a man fed 2,2-dimethyl[1- ^{14}C]stearic acid. Phase system C. Solid line: alkaline consumption. Broken line: radioactivity.

The identity of the active material in the second peak of Fig. 2 is not yet established. When part of the butanol extract of the first day urine was chromatographed according to system C the picture shown in Fig. 4 was obtained. The second active band corresponds to 2,2-dimethylsuccinic acid. The front band was hydrolysed with alcoholic KOH at 120° for 6 hours. When the acids were extracted and chromatographed with solvent system B the radioactivity appeared at the positions of 2,2-dimethyladipic acid and 2,2-dimethylsuccinic acid.

The feces were collected in ethanol. They were homogenized and saponified, and the fatty acids were extracted with ether from the acidified aqueous mixture. Most of the fecal activity appeared during the second plus third day (cf. Table 2).

Discussion

The results of this investigation are in general agreement with those obtained in animal experiments (1). 2,2-Dimethylstearic acid was well absorbed from the intestine. The analyses of the lymph lipids showed that the 2,2-dimethylstearic acid

N. TRYDING, 2,2-Dimethylstearic acid

was mainly incorporated into the neutral fat but it was also found in the phospholipids and in the free form.

The main metabolite of 2,2-dimethylstearic acid was 2,2-dimethyladipic acid, which was excreted in the urine, but also 2,2-dimethylsuccinic acid, which was not isolated from the urine of rats (1), was identified. The acids were found to be excreted partly in the conjugated form.

The lymph fat did not contain any short dicarboxylic acids as judged from the chromatograms with phase system B. Presumably the degradation of 2,2-dimethylstearic acid to 2,2-dimethyladipic and 2,2-dimethylsuccinic acid does not take place in the intestinal mucosa. The liver is the most probable site of the oxidizing enzymes involved.

SUMMARY

2,2-Dimethylstearic acid was well absorbed when fed orally to humans. It was found to be incorporated into the glycerides and phospholipids of the lymph but also existed in unesterified form.

After the administration of inactive dimethylstearic acid to a human subject, dimethyladipic acid could be isolated from the urine. This finding was confirmed with 2,2-dimethyl[1-¹⁴C]stearic acid. The main metabolite was 2,2-dimethyladipic acid, but in addition 2,2-dimethylsuccinic acid was identified.

The author is greatly indebted to Professor Haqvin Malmros, Department of Internal Medicine, University of Lund, for the facilities placed at his disposal, and to Dr. Gunnel Westöö for the preparation of the dimethylstearic acid used in this work.

Thanks are due to Dr. Arne Munke who made the thoracocentesis and to Miss Kerstin Sjöström who was the technical assistant.

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REFERENCES

1. BERGSTRÖM, S., BORGSTRÖM, B., TRYDING, N., and WESTÖÖ, G., *Biochem. J.* 58, 604 (1954).
2. TRYDING, N., and WESTÖÖ, G., *Acta Chem. Scand.* 11, 427 (1957).
3. BLOMSTRAND, R., *Am. J. Med.* To be published.
4. BORGSTRÖM, B., *Acta Physiol. Scand.* 25, 101 (1952).
5. BORGSTRÖM, B., *Acta Physiol. Scand.* 25, 111 (1952).

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Uppsala 1957. Almqvist & Wiksells Boktryckeri AB

On the metabolism of 2,2-dimethylnonadecanoic acid in man

By NILS TRYDING and GUNNEL WESTÖÖ

With 4 figures in the text

2,2-Dimethylnonadecanoic acid has earlier been fed to rats (Tryding and Westöö, 1). As the α -carbon atom is quaternary, no α , β -dehydrogenation could take place, and the molecule could not be β -oxidized from the carboxyl end. The main metabolic end products were excreted in the urine and were found to be 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid. Presumably the last step of the degradation from the ω -end had been retarded by the steric hindrance of the two methyl groups.

An opportunity to study the metabolism of 2,2-dimethylnonadecanoic acid in man was given in connection with an investigation on the extent of hydrolysis of glyceride ester bonds in the human intestine (2). In this study glycerides containing 2,2-dimethyl[1- 14 C]nonadecanoic acid were fed to a human subject. The urine was collected, and the main labelled compounds excreted were isolated with the technique used in the animal experiments (1).

Experimental

The acids used are described earlier (1). The specific activity of the 2,2-dimethyl[1- 14 C]nonadecanoic acid was 0.15 μ C/mg, or 125,000 cpm/mg when counted in a "Tracerlab" gas-flow counter. The chromatographic separations were performed by the methods described in earlier papers (1, 3). The following phase systems were used:

Stationary phase	Moving phase
B 2-ethylhexanol/chloroform 1/1	methanol/water 2/3
C butanol	water

The supporting medium for the stationary phases (8 ml) was hydrophobic Hyflo supercel (9 g).

Results

About 20 mg of 2,2-dimethyl[1- 14 C]nonadecanoic acid were transesterified with corn oil and fed in a test meal (cf. 2) to a healthy male student. The urine from the first 6 hours after the administration of the labelled glycerides was acidified with hydrochloric acid and extracted with ether. The extract contained 40,000 cpm,

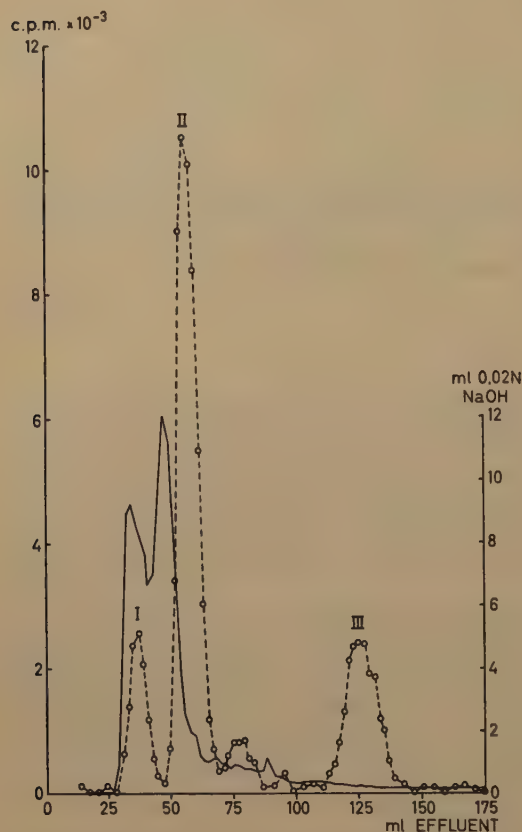


Fig. 1. Partition chromatography of an ether extract of the first-day urine. Phase system B. Solid line: alkali consumption. Broken line: radioactivity.

i.e., about 6 per cent of the absorbed radioactivity, which was approximately calculated as the fed activity less the activity recovered in the feces and in the specimens of intestinal contents taken during the experiment (2). Another urine sample was collected during the next 18 hours. After acidification it was extracted with ether and butanol. The activity of the ether extract was found to be 180,000 cpm, while 130,000 cpm were recovered in the butanol extract. Determinations of radioactivity were also performed on the extracts of the urine from the third and fourth days (cf. Table 1).

Table 1. Radioactivity recovered in the urine after oral administration of glyceride esters of 2,2-dimethyl[1- 14 C]nonadecanoic acid.

Day no.	Per cent of absorbed activity found in the urine
1	53
2	24
3	12
4	3

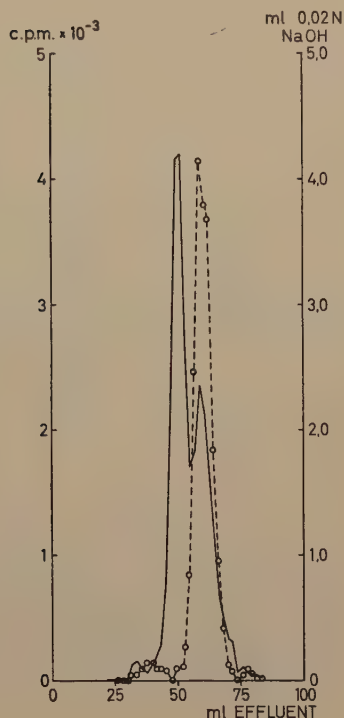


Fig. 2. Identification of 2,2-dimethylglutaric acid. Phase system B. Solid line: alkali consumption. Broken line: radioactivity.

Part of an ether extract of the second urine sample was chromatographed with solvent system B. As shown in Fig. 1 three distinct active peaks were found. The acids of the second active band were rechromatographed together with inactive 2,2-dimethylglutaric acid (9 mg) with the same phases as earlier. The titration curve of the added dicarboxylic acid was found to coincide with the radioactivity curve (Fig. 2). The first of the two titration peaks was formed by ordinary urine excretion products. When the material of the radioactive peak was rechromatographed on a 4.5 g column according to phase system C, the titration and radioactivity peaks were likewise found at the same position (Fig. 3). The identity of the labelled metabolite with 2,2-dimethylglutaric acid was confirmed by five repeated recrystallizations with added carrier from hydrochloric acid and benzene-light petroleum alternately.

Part of the material of the third active band in solvent system B (cf. Fig. 1) was rechromatographed together with synthetic, unlabelled 2,2-dimethylpimelic acid (3.7 mg). The chromatogram is shown in Fig. 4. Five crystallizations were performed with the labelled material and inactive 2,2-dimethylpimelic acid, from water and from benzene-light petroleum alternately. The specific activities of the crystals remained constant, indicating the identity of the radioactive compounds with the added carrier. The most polar radioactive product of the ether extract (peak I, Fig. 1) has not yet been identified.

When a butanol extract of the urine was chromatographed according to phase system C, the main radioactive peak was found at the front. The material of this

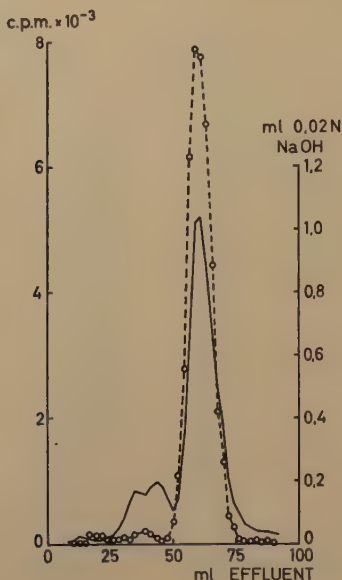


Fig. 3. See Fig. 2. Phase system C (4.5 g column).

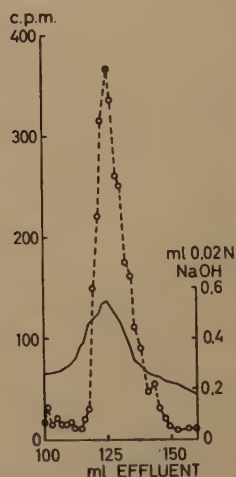


Fig. 4. Identification of 2,2-dimethylpimelic acid. Phase system B. Solid line: alkali consumption. Broken line: radioactivity.

peak was hydrolysed with alcoholic KOH at 120° for 6 hours and rechromatographed with phase system B. The activity appeared at the positions of 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid, indicating that these acids were partly excreted in the conjugated form.

SUMMARY

2,2-Dimethylnonadecanoic acid has been fed to a human subject. The main metabolites were 2,2-dimethylglutaric acid and 2,2-dimethylpimelic acid, which were excreted in the urine. These results are in accord with those of earlier animal experiments.

The technical assistance of Miss Kerstin Sjöström is gratefully acknowledged.

This work is part of investigations supported by *Knut och Alice Wallenbergs Stiftelse* and by the *Medical Faculty of the University of Lund*.

Department of Physiological Chemistry, University of Lund, Lund, Sweden.

REFERENCES

1. TRYDING, N., and WESTÖÖ, G., *Acta Chem. Scand.* **10**, 1234 (1956).
2. BORGSTRÖM, B., TRYDING, N., and WESTÖÖ, G., *Acta Physiol. Scand.* In press (1957).
3. BERGSTRÖM, S., BORGSTRÖM, B., TRYDING, N., and WESTÖÖ, G., *Biochem. J.* **58**, 604 (1954).

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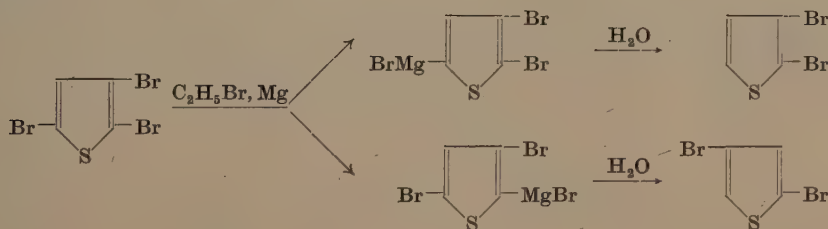
Thiophene chemistry. Part II¹

A new method of preparing 2,4-dibromothiophene and its Grignard and lithium reagents. Two-stage mechanism in the metalation of 2,4-dibromothiophene with butyllithium

By SVEN-OLOV LAWESSON

With 4 figures in the text

In connection with work on thiophenebromo derivatives it was of importance to work out a new and better method for the synthesis of 2,4-dibromothiophene and thus make the compound readily available. Already in 1934 Steinkopf and coworkers (1) claimed to have prepared 2,4-dibromothiophene in a pure state. Starting from 2,3,5-tribromothiophene they prepared the Grignard reagent by "entrainment" according to:



By hydrolyzing the Grignard reagents they obtained a mixture of 2,3-dibromo- and 2,4-dibromothiophene, the second isomer being in an exceedingly low yield, however. Because the boiling points were almost identical, the separation by distillation was impossible. As the melting points differed by about 10–15 degrees, fractional crystallization of 2,3-dibromothiophene was first performed and from the mother liquor the $HgCl$ compound was then precipitated. This latter mercury compound was claimed to be exclusively the 2,4-derivative, a conclusion which does not seem to be correct. The purity of the isomer must undoubtedly be taken with reservation.

The preparation of 2,4-dibromothiophene via the Grignard reagent is not a recommendable method and should perhaps be abandoned. The Grignard reagent is difficult to prepare and an auxiliary halide must be used. Furthermore almost nothing is known about the mechanism of entraining agent. Thus it is not possible

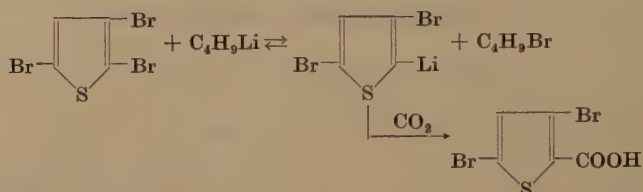
¹ Reference 3 is considered as Part I of this series.

to predict in what α -position the magnesium will enter and as the reaction time is long and the reaction sluggish one may expect the selectivity to be small and side reactions may predominate.

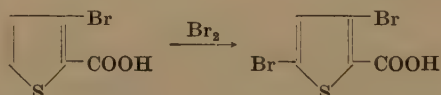
Steric hindrance from the bromine in the 3-position may also play an important role, and as a logical consequence of this it may be expected that the 2,3-dibromo isomer will predominate. This has indeed been confirmed by Steinkopf's experiments.

Bromination of 2-bromothiophene with one mol of bromine gave only 2,5-dibromothiophene (2) and if 3-bromothiophene (2, 3), now readily available, was treated with one mol of bromine, 2,3-dibromothiophene exclusively was formed (2).

In the first paper of this series (3), which was of a preliminary character, 3,5-dibromo-2-thiophenecarboxylic acid was prepared according to:



The identity of the product was established with the help of differences in the infrared spectrograms and melting points. That the lithium-bromine interconversion will appear at the 2-position at low temperatures has now also been shown by bromination of 3-bromo-2-thiophenecarboxylic acid in glacial acetic acid. Analogous to the bromination of 2-thiophenecarboxylic acid, the hetero atom, sulfur, controls the substitution, and the meta-directing carboxylic acid group has no influence, i.e. the bromine enters the 5-position rather than the 4-position. The result of the bromination of 3-bromo-2-thiophenecarboxylic acid is:



The identity of the product was confirmed by infrared analysis, too.

By hydrolyzing the 3,5-dibromo-2-thiophenelithium reagent, prepared at -40°C , with water, we obtained 2,4-dibromothiophene in a good yield. Its infrared spectrum has been compared with that of 2,3-dibromothiophene (2) and from them it was evident that the 2,4-dibromothiophene is free from the other isomer.

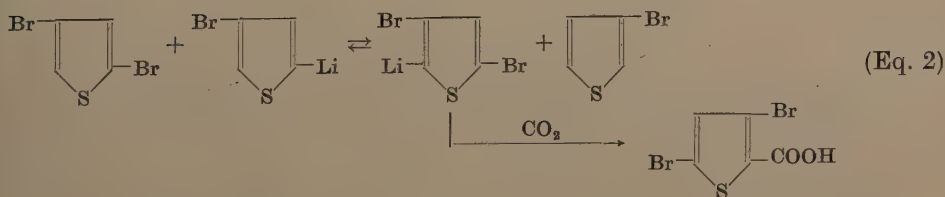
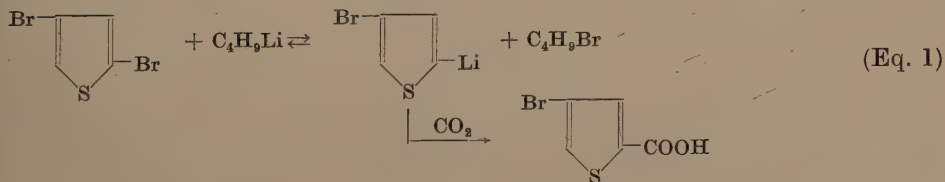


That butyllithium attacks the 2-position is an indication that the mechanism is nucleophilic. The inductive effect ($-I$) plays the most important role in interconversion with lithium reagents and the bromine in the 3-position pulls electrons from the carbon to which it is attached, thus making the carbon in the 2-position positive.

If the halogen-lithium exchange reaction was performed at room temperature and for a longer time a mixture of 3,5-dibromo and 4,5-dibromo-2-thiophenecarboxylic acids was obtained after carboxylation. No attempts were made to determine the isomer ratio and it is no use speculating without experimental evidence on this subject. However, it is worth mentioning that because the organo-lithium reagents are very reactive, compared to the Grignard reagents (4), side reactions can predominate and must be avoided. The types of bonds in the reactants and the products are the same in principle, the accompanying free energy changes will be small and consequently not one but several equilibria must exist when we treat 2,3,5-tribromothiophene, a compound having two active positions, with butyllithium. That the first step, at least at low temperature, is an attack at the 2-position is shown by the fact that 3,5-dibromo-2-thiophenecarboxylic acid is isolated. The exact details of the whole reaction are at present unknown.

Steinkopf *et al.* did not succeed in preparing the Grignard reagent from 2,4-dibromothiophene. It failed to react with magnesium alone in ether and in this respect it also differed markedly from e.g. *p*-dibromobenzene (5) which gave both mono- and di(magnesium halides). We have, however, been able to bring 2,4-dibromothiophene into reaction with magnesium in ether by using ethyl bromide as entraining agent and have studied the effect on the yields of different amounts of ethylbromide (1–5 mol). Optimal yields of 4-bromo-2-thiophenemagnesium bromide, assessed by the carboxylation method, were obtained by the use of 5 mol of ethyl bromide, 4-bromo-2-thiophenecarboxylic acid being then obtained in a yield of 51 per cent. The Urion mechanism (6) of entrainment which is stressed by the results, will be discussed in a subsequent paper (7).

By treating 2,4-dibromothiophene at -60°C with one mol of butyllithium and then following this with carboxylation, 4-bromo-2-thiophenecarboxylic acid was obtained. However, at room temperature we obtained in the same way after a short reaction time 3,5-dibromo-2-thiophenecarboxylic acid. This is in fact very surprising because metalation of a cyclic sulfide containing a bromine atom ortho to the hetero atom has not hitherto been observed. We propose the following two-stage mechanism:



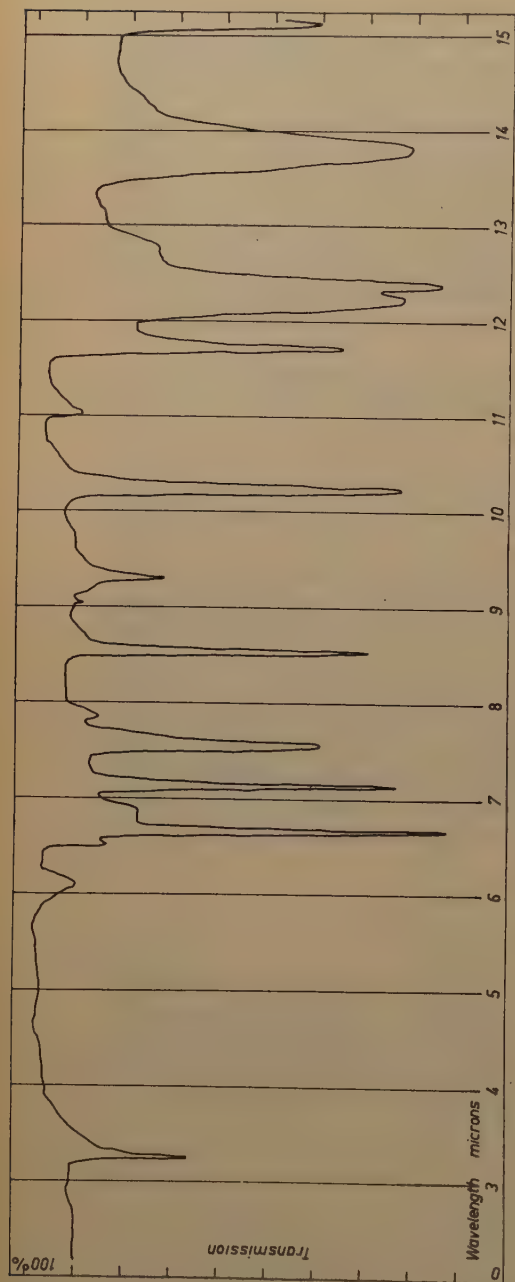
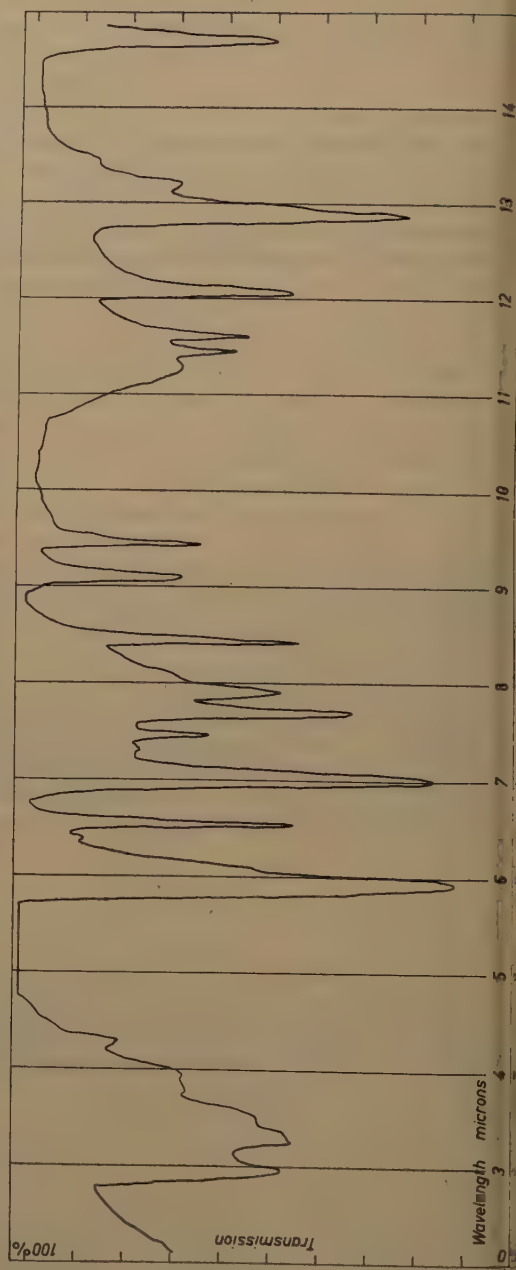


Fig. 1. The infrared spectrum of 2,4-dibromothiophene (liq.).



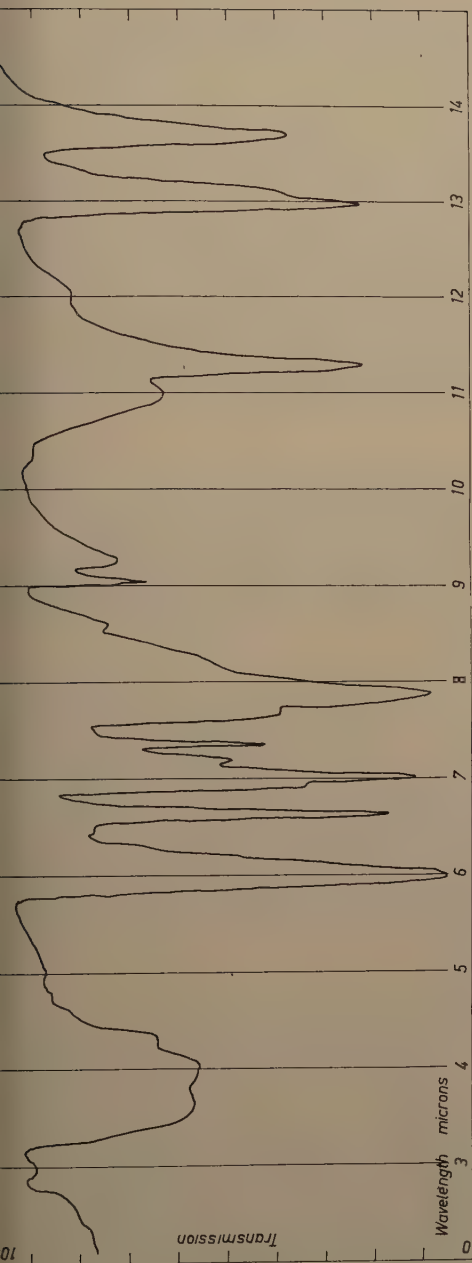


Fig. 3. The infrared spectrum of 3-bromo-2-thiophenecarboxylic acid (in pressed KBr).

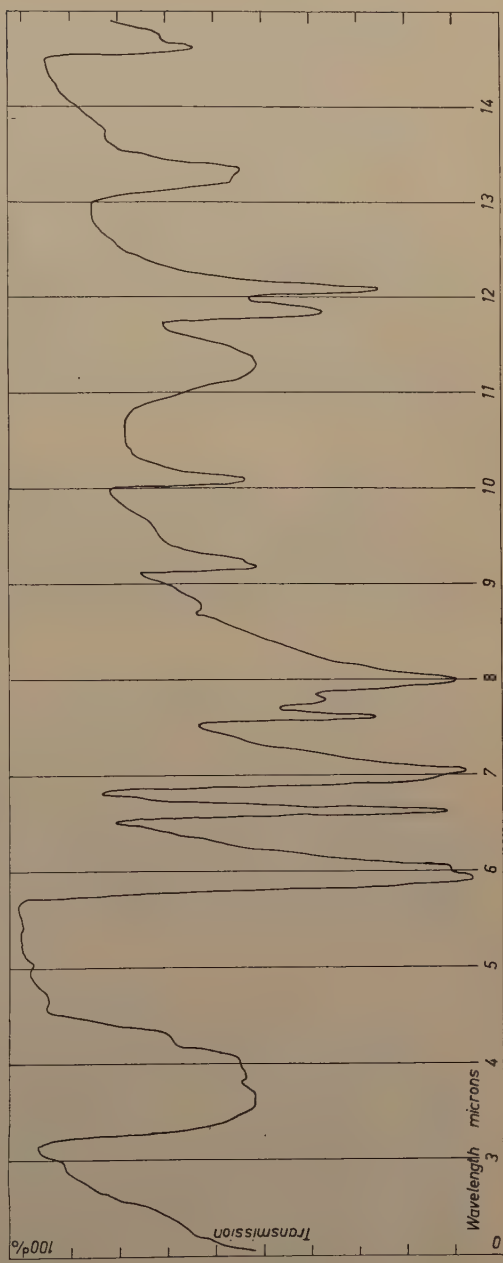


Fig. 4. The infrared spectrum of 3,5-dibromo-2-thiophenecarboxylic acid, prepared by metalation of 2,4-dibromothiophene (in pressed KBr).

At a low temperature the first stage is undoubtedly lithium-bromine interconversion at the 2-position (Eq. 1). At room temperature the picture is more complicated.



Either butyllithium can metalate 2,4-dibromothiophene directly which, however, seems to be highly improbable as the yield is only 28 per cent, or also the first formed 4-bromo-2-thiophenyl lithium reagent metalates 2,4-dibromothiophene faster than the latter undergoes lithium-bromine exchange (Eq. 2). It is possible, too, that the β -bromine, by its strong inductive effect, makes the α -carbon in the 5-position less negative than those in the other positions and that this effect only plays an important role at room temperature—e.g., that at higher temperatures the metalation of 2,4-dibromothiophene is a faster reaction than lithium-halogen interconversion.

Experimental

All experiments with lithium and magnesium reagents were performed in a common Grignard apparatus under nitrogen atmosphere. The infrared spectra were recorded with a Perkin Elmer Spectrophotometer (model 21) equipped with NaCl optics.

Preparation of 2,4-dibromothiophene

To a solution of 32 g 2,3,5-dibromothiophene (2) in 75 ml of absolute ether, cooled to -40°C , 140 ml 0.7 *M* butyllithium (in ether) was added very quickly. After stirring for four minutes the solution was poured into cold water. The organic layer was separated, the water phase was shaken with ether and after the combined extracts had been shaken with water they were dried over sodium sulphate. The solution was concentrated and then distilled, giving as the main fraction, 2,4-dibromothiophene. B.p. $83\text{--}85^{\circ}\text{C}/9\text{--}10$ mm Hg. Weight 18.1 g. Yield 75%. The infrared spectrum, Fig. 1.

Preparation of 4-bromo-2-thiophenecarboxylic acid by "entrainment"

In a suitable flask 8 g of magnesium was covered with 50 ml of absolute ether. A solution of 12 g of 2,4-dibromothiophene and 28 g of ethylbromide in 75 ml of dry ether was run in at such a rate that gentle reflux existed. The mixture was heated under reflux for 20 hours and then after cooling poured on solid carbon dioxide in ether. After the Dry Ice had evaporated, the mixture was hydrolysed with water; the organic layer was separated, the water solution extracted with ether and the combined ether layers extracted with dilute sodium hydroxide. Upon acidification of the alkaline solution a white powder precipitated; this after recrystallisation from water-ethanol gave small fine plates. M.p. $122\text{--}124^{\circ}\text{C}$. Weight 5.1 g. Yield 51%.

Anal. Subst. 51.47 mg; 4.87 ml of 0.05106 *M* NaOH.

$\text{C}_5\text{H}_3\text{BrO}_2\text{S}$: Calc. Equiv. wt. 207.1.

Found Equiv. wt. 206.9.

Preparation of 4-bromo-2-thiophenecarboxylic acid via the lithium reagent

To a solution of 12.1 g of 2,4-dibromothiophene in 75 ml of absolute ether at -70°C 56 ml of 0.9 *M* butyllithium was added in a fast stream during 0.5 min. The solution was stirred for another three minutes and then treated at once with an excess of powdered solid carbon dioxide in ether and worked up as above. M.p. and mixed m.p. $122-124^{\circ}\text{C}$. Weight 7.1 g. Yield 69%. Infrared spectrum, Fig. 2. The infrared spectrum of 3-bromo-2-thiophenecarboxylic acid (3), Fig. 3.

4-Bromo-2-thiophenecarboxylic amide

White plates from water-ethanol. M.p. $148-150^{\circ}\text{C}$.

Anal. Subst. 24.11 mg; 26.1 mg of CO_2 ; 4.37 mg of H_2O .

$\text{C}_5\text{H}_4\text{BrOSN}$ (206.09): Calc. C 29.14, H 1.97.

Found C 29.42, H 2.03.

Metalation of 2,4-dibromothiophene

To a solution of 2.4 g of 2,4-dibromothiophene in 20 ml of absolute ether 10 ml of 1 *M* butyllithium was added in a slow stream at room temperature. The solution was stirred for 10 minutes and was then carboxylated and worked up as above. Faintly yellow-coloured needles from water-ethanol. M.p. and mixed m.p. $210-211^{\circ}\text{C}$. Weight 0.79 g. Yield 28%. Infrared spectrum, Fig. 4.

Anal. Subst. 50.31 mg; 3.58 ml of 0.0495 *M* NaOH.

$\text{C}_5\text{H}_2\text{Br}_2\text{O}_2\text{S}$. Calc. Equiv. wt. 285.95.

Found Equiv. wt. 284.0.

Bromination of 3-bromo-2-thiophenecarboxylic acid

2 g of 3-bromo-2-thiophenecarboxylic acid was dissolved in 30 ml of glacial acetic acid. 1.8 g of bromine in 10 ml of glacial acetic acid was run in. After stirring for 10 hours the solution was poured into ice water. The precipitate was filtered and recrystallized from ethanol-water. Faintly yellow needles. M.p. and mixed m.p. $210-212^{\circ}\text{C}$.

Anal. Subst. 61.47 mg, 4.20 ml of 0.05106 *M* NaOH.

$\text{C}_5\text{H}_2\text{Br}_2\text{O}_2\text{S}$: Calc. Equiv. wt. 285.95.

Found Equiv. wt. 286.6.

SUMMARY

2,4-Dibromothiophene has been prepared from 2,3,5-tribromothiophene by debromination with butyllithium at a low temperature.

The mono Grignard reagent has been formed from 2,4-dibromothiophene by using ethylbromide as entraining agent and the corresponding acid has been prepared.

The 4-bromo-2-thiophenelithium reagent has been shown to be very reactive. It will form 4-bromo-2-thiophenecarboxylic acid at low temperature. At room temperature 2,4-dibromothiophene is metalated either by the butyllithium or by the 4-bromo-2-thiophenelithium reagent.

ACKNOWLEDGEMENTS

To Professor A. Fredga I wish to express my sincere gratitude for his interest and for valuable discussions. Thanks are also due to Dr. A. Rosenberg and Mr. S. Bergwall for taking the infrared absorption curves.

The investigation has been partly supported by a grant from the *Swedish Natural Science Research Council*, which is gratefully acknowledged.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, February 1957.

REFERENCES

1. STEINKOPF, W., JACOB, H., and PENZ, H., *Ann.* 512 (1934), 136.
2. LAWESSON, S.-O., *Arkiv Kemi* (1957), part VI (to be published).
3. LAWESSON, S.-O., *Acta Chem. Scand.* 10 (1956), 1020.
4. SWAIN, C. G., *J. Am. Chem. Soc.* 69 (1947), 2306.
5. GILMAN, H., BEABER, N. J., and JONES, H. L., *Rec. trav. chim.* 48 (1929), 597.
6. URION, E., *Compt. rend.* 198 (1934), 1244.
7. LAWESSON, S.-O., *Arkiv Kemi* (1957), part IV, 11, 337.

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Thiophene chemistry. Part III

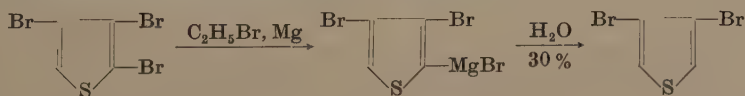
On the preparation of 3,4-dibromothiophene and its Grignard and lithium reagents. Two-stage metalation of 3,4-dibromothiophene. Substitution reactions of 4-bromo-3-thiophenecarboxylic acid

By SVEN-OLOV LAWESSON

With 4 figures in the text

In 1934 Steinkopf and his coworkers (1) carried out the first systematic investigation of isomers of bromothiophenes. Since that time very little work has been performed in this field and in fact, the methods of Steinkopf were very troublesome and time-consuming and the yields often very bad. Consequently it is of interest to make these bromothiophene derivatives more easily available as aromatic bromoderivatives are indispensable starting compounds for the preparation of the important Grignard reagents and the highly reactive corresponding lithium compounds. During the last three decades the latter have assumed considerable importance as intermediates. In practical importance there is much rivalry between the two and although the Grignard reagents are still the most widely used, the organo-lithium reagents can be formed from a greater range of organic structures. In thiophene chemistry the lithium reagents are even more important than the Grignard compounds because of difficulties in the preparation of the latter (2).

Steinkopf *et al.* (1) prepared 3,4-dibromothiophene by hydrolyzing the Grignard reagent from 2,3,4-tribromothiophene according to:

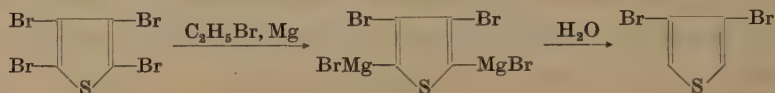


Still poorer yields were obtained if they started from 2,3,4,5-tetrabromothiophene. This is, however, comprehensible from the fact, that it is remarkably difficult to form two -MgX groups in the same aromatic kernel. For instance a dimagnesium derivative (3,4,5) was formed from the interaction of *p*-dibromobenzene only under suitable conditions and 1,4-dibromonaphthalene behaved in the same way (6). Of the three diiodobenzenes only the meta and para isomers gave dicarboxylic acid (15 and 50 % yield respectively) (7,8) while the ortho isomer gave by-products only. There is only one known case where an *o*-di-MgX compound was produced (9), viz.



This is very surprising but the 3- and 4-positions in the thiophene kernel may perhaps not be "true" ortho positions.

At this Institute we have first tried to prepare 3,4-dibromothiophene, starting from tetrabromothiophene, according to the reaction:

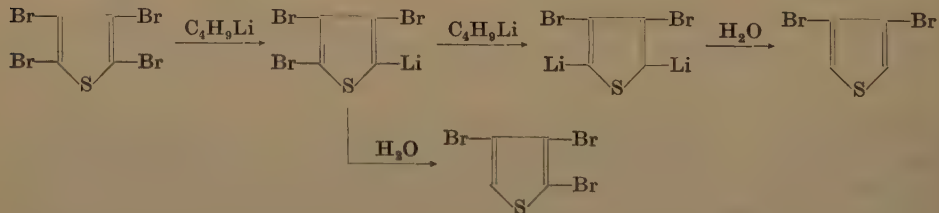


To prepare the Grignard reagent is impossible without an auxiliary halide but by using ethyl bromide as "entrainer" the yields have been raised with the ratio of entraining agent to thiophene halide. From Table 1 this is clear:

Table 1. Preparation of 3,4-dibromothiophene by hydrolyzing 3,4-dibromo-2,5(dimagnesiumbromide)-thiophene.

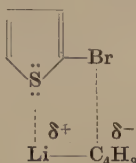
2,3,4,5-tetra bromothiophene (mol)	Ethyl bromide (mol)	Yields of 3,4-dibromo- thiophene (%)
0.1	0.1	9
0.1	0.3	20
0.1	0.5	29

By using butyllithium as dehalogenation agent (10, 11) instead of the Grignard reagent the yields will be higher. Whether we used one or two mol of butyllithium per mol reactant 2,3,4-tribromothiophene and 3,4-dibromothiophene respectively were obtained according to



There is no doubt that isomer-free products were obtained by this method as it is well known that lithium-halogen exchange always occurs at a halogen ortho to a hetero atom (or substituent) with an unshared electron pair. About the mechanism we hitherto know nothing with certainty but it is believed to resemble

the lithium metalation reaction. According to this the first step is a coordination between the metal cation and a pair of unshared electrons of the hetero atom (or substituent). For lithium-bromine exchange the following is valid:



From dipole moment data (12) it is indicated that lithium alkyls should be regarded as covalent compounds with about 45 per cent ionic character. Conductometric measurements (13) show that neither conduction nor transference occurred with simple organolithium compounds. From the above picture one is forced to believe that an alkyl lithium reagent appears to be freely ionic. This statement should not be interpreted as implying that actual dissociation of the reagent into ions is a necessary prelude to reaction. It is sufficient to postulate that heterolytic scission of the C_4H_9-Li bond occurs in the course of the reaction.

The next step in the lithium-bromine exchange reaction is uncertain and not thoroughly investigated. Morton (14) claims the attack to be electrophilic, Roberts and Curtin (15) and Sunthakar and Gilman (16) propose a nucleophilic mechanism and although each has some merit none is completely satisfactory. Both Morton (17) and Gilman (18) say, that "the electrophilic and nucleophilic interpretations of metalation are seen as limiting extremes, with some individual reactions lying nearer one extreme than to the other". In respect to the problem of lithium-halogen exchange, Marton's view that the addition reagents are to be regarded as electrophilic seems extreme. This will be dealt with in a later paper too (19). Quite recently (20) Bryce-Smith has published results, that give evidence for homolytic reactions between alkyl-lithium compounds and alkyl halides.

4-Bromo-3-thiophenecarboxylic acid has earlier been prepared by Steinkopf *et al.* (1) but no details about the procedure and yield were given. We have here tried to make the mono-Grignard reagent of 3,4-dibromothiophene without entraining agent but without success. The reactivity of β -substituted bromothiophenes in the Grignard reaction is exceedingly low (compare 3-bromothiophene (1)) and when, as in this case, we have an ortho-dibromothiophene, it will be still more difficult to prepare the reagent.

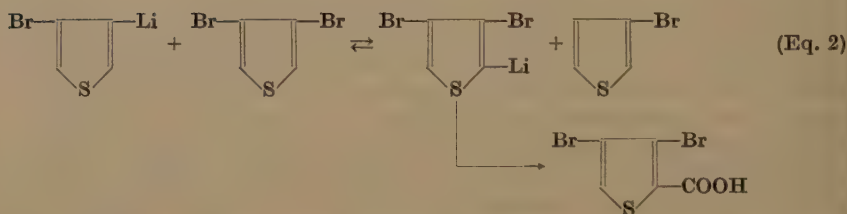
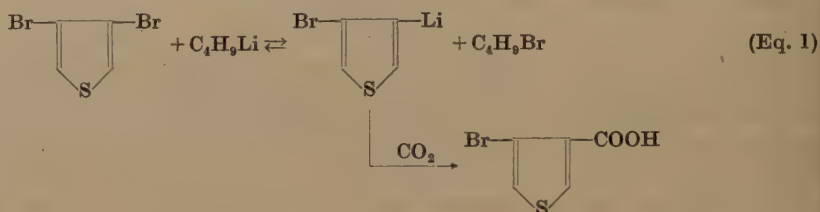
However, by using ethyl bromide as "entrainer" we could isolate the desired acid and as in the formation of Grignard reagent from tetrabromothiophene, we observed an increased yield on increasing the proportion of ethyl bromide to thiophene halide. Thus the yields were 10, 24 and 38 per cent when the ratios between entraining agent and halide were 1/1, 3/1, and 5/1 respectively.

The preparation of 4-bromo-3-thiophenecarboxylic acid could be performed in a better way by using the halogen-lithium exchange method. The lithium atom is much smaller than that of magnesium, so steric hindrance must be slight. However, the lithium reagents are more reactive than the magnesium compounds and this may complicate the synthesis. The lithium-halogen exchange is an equilibrium reaction



and when the strongly electropositive lithium has a tendency to become attached to the most electronegative group, it is necessary to know the electronegativities of and choose the right R and R' groups (22,23,24,25). Also the new organolithium reagent RLi will enter into side reactions especially if the reaction period is long and the temperature moderate. If RLi is an aromatic lithium compound it may under proper conditions prefer metalation to halogen interconversion and thus, see the equation above, RLi will metalate the original reactant RX. The reason may be differences in electronegativities among other things.

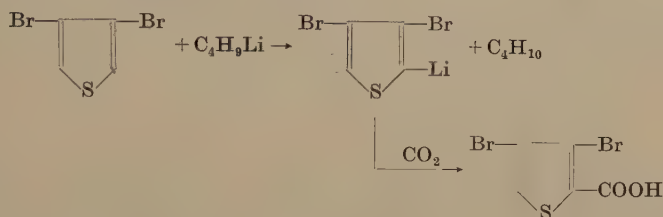
4-Bromo-3-thiophenecarboxylic acid was prepared in a good yield by treating 3,4-dibromothiophene with butyllithium for a few minutes at a low temperature (-70°C) followed by carboxylation (Eq. 1).



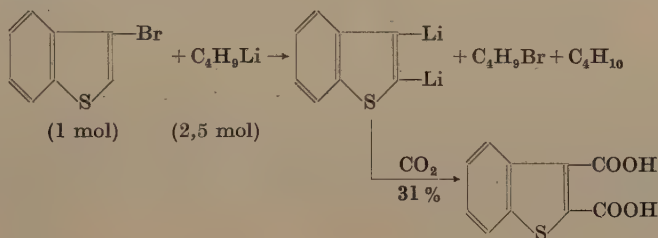
By treating the reactant with butyllithium for a longer time and at room temperature 3,4-dibromo-2-thiophenecarboxylic acid was isolated. The most plausible explanation of this is, because the lithium-halogen exchange is momentary, that the 4-bromo-3-thiophenelithium reagent formed, which must be highly reactive, can metalate 3,4-dibromothiophene faster than the halogen-lithium interconversion occurs (Eq. 2). We have also found, that if a great excess of butyllithium was used and the procedure was carried out very fast and at room temperature, 4-bromo-3-thiophenecarboxylic acid was formed. The yield was, however, not especially high and if we prolonged the reaction time both acids were obtained in a mixture, because halogen-lithium interconversion and metalation occurred at the same time.

Two-stage mechanisms in organo-lithium chemistry are in no way unique (25), but no explanation of this phenomenon has yet been made. As to the second stage it has been pointed out that aromatic lithium reagents have a stronger tendency than alkyl lithium compounds to metalate. However, from work of Wittig and coworkers (26), who used phenyllithium and Gilman *et al.* (27, 28) who used butyllithium no significant difference could be observed. In fact it has been found, that the yield of metalation products of dibenzofuran (29) under comparable conditions in diethyl ether decreases according to the following order: *n*-butyl-

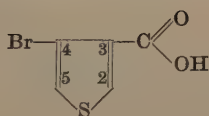
lithium > ethyllithium > *n*-amyllithium > phenyllithium > methyllithium. The hypothetical two-stage mechanism above or any other for that matter cannot thus with certainty be accepted. At low temperature we can be sure that only bromine-lithium interconversion occurs. At room temperature the formation of 3,4-dibromo-2-thiophenecarboxylic acid can be explained by the two-stage mechanism above or by a parallel "Zerewitinoff" reaction



And this is because the bromine atoms in the β -positions by their strong inductive effect make the α -hydrogens "acidic". Wittig (30) has also earlier pointed out that the inductive effect ($-I$) predominates over the tautomeric effect in metalation with lithium reagents. That such a parallel reaction is possible is further indicated by the formation of thianaphthene-2,3-dicarboxylic acid (31) from 3-bromothianaphthene according to:

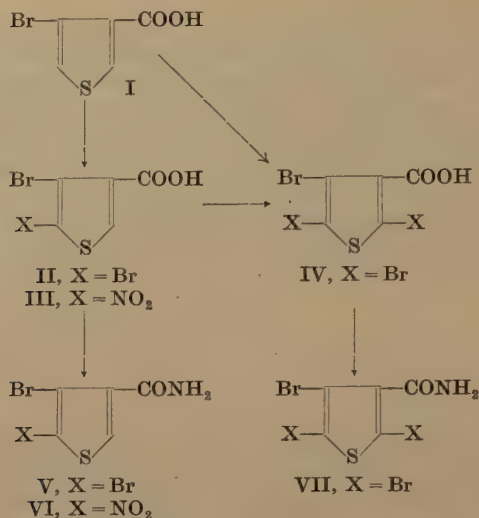


Substitution reactions of 4-bromo-3-thiophenecarboxylic acid have hitherto not been performed



Both the bromine atom which is ortho-directing with deactivation and the carboxylic group which is meta-directing with deactivation will in electrophilic substitution make the 5-position most negative and consequently the first substituent will enter there. The second one will enter the 2-position notwithstanding its being ortho to the carboxylic group but owing to the high reactivity of the α -positions in thiophene. However, some decarboxylation was observed with formation of tetrabromothiophene.

Bromination of 4-bromo-3-thiophenecarboxylic acid (I) has been performed with one mol of bromine in glacial acetic acid and 4,5-dibromo-3-thiophenecarboxylic acid (II) was thus obtained. 2,4,5-Tribromo-3-thiophenecarboxylic acid (IV) was formed by bromination of II with excess of bromine or directly from I. The acids I, II and IV were transformed to their amides and characterized. See Scheme 1.



Scheme 1.

Nitration of 4-bromo-3-thiophenecarboxylic acid (I) with mixed acids gave 4-bromo-5-nitro-3-thiophenecarboxylic acid (III) which was then characterized as its amide (VI). Further nitration to a dinitro derivative was impossible because the introduction into an aromatic kernel of two meta-directing groups in ortho-positions generally is improbable by direct methods. However, in thiophene chemistry it is for instance possible to nitrate 2,5-dibromothiophene (32) to 2,5-dibromo-3,4-dinitrothiophene. This indicates further that the 3- and 4-positions in thiophene differ from real ortho-positions.

Experimental

All preparations and reactions of Grignard- and lithium reagents were carried out in a common Grignard apparatus and in nitrogen atmosphere. The infrared spectra were recorded on a Perkin-Elmer spectrophotometer (model 21) equipped with NaCl optics.

Preparation of 3,4-dibromothiophene via the Grignard reagent

15 g of magnesium was placed in a flask and covered with 75 ml of dry ether. A mixture of 40 g of tetrabromothiophene and 55 g of ethyl bromide in 100 ml of absolute ether was run in at such a rate that the reaction did not stop. When the addition was complete the flask was heated under reflux for 14 hours. After cooling, the ether phase was poured out into a dilute solution of hydrochloric acid, the organic phase separated and the water phase shaken a few times with water. The combined extracts were shaken free from acid, dried over sodium sulphate, freed from solvents and distilled.

Fraction 1. 3,4-dibromothiophene. B.p. 93–95°C/10 mm Hg. Weight 7 g. Yield 29 %.

Fraction 2. 2,3,4-tribromothiophene. B.p. 132–134°C/10 mm Hg. Weight 14.1 g. Yield 44 %.

Preparation of 3,4-dibromothiophene via the lithium reagent

To a solution of 47 g of 2,3,4,5-tetrabromothiophene in 150 ml of absolute ether, cooled to 0°C in an ice water mixture, 250 ml of 1 *M* butyllithium (in ether) was added during 60 minutes. After stirring for 15 minutes the solution was poured into cold water. The organic layer was separated, the water phase shaken with ether and after the combined extracts had been shaken with water, they were dried over sodium sulphate. The solution was concentrated and then distilled, giving as the main fraction, 3,4-dibromothiophene. B.p. 93–95°C/10 mm Hg. Weight 21 g. Yield 74 %. The infrared spectrum, Fig. 1.

Preparation of 4-bromo-3-thiophenecarboxylic acid by "entrainment"

In a flask was placed 8 g of magnesium which was covered with 50 ml of absolute ether. A solution of 12 g of 3,4-dibromothiophene and 28 g of ethyl bromide in 75 ml of dry ether was run in at such a rate that gentle reflux existed. The mixture was heated under reflux for 19 hours and then after cooling poured on to solid carbon dioxide in ether. After the Dry Ice had evaporated the mixture was hydrolyzed with water, the organic layer separated, the water solution extracted with ether and the combined ether layers extracted with dilute sodium hydroxide. Upon acidification of the alkaline solution a white powder precipitated; this, after recrystallization from water-ethanol solution, yielded small fine needles. M.p. 157–159°. Weight 3.9 g. Yield 38 %.

Preparation of 4-bromo-3-thiophenecarboxylic acid via the lithium reagent

To a solution of 12.1 g of 3,4-dibromothiophene in 75 ml of absolute ether, at a temperature of –70°C, 45 ml of 1.1 *M* butyllithium was added in a fast stream during 0.5 minutes. The solution was stirred for another 2.5 minutes and then treated at once with an excess of powdered solid carbon dioxide in ether and worked up as above. M.p. and mixed m.p. 157–159°. Weight 7.5 g. Yield 73 %. Infrared spectrum, Fig. 2.

4-Bromo-3-thiophenecarboxylic amide

About 1 g of I was refluxed with an excess of thionyl chloride for 45 minutes. The excess of SOCl₂ was evaporated and the crude carboxylic chloride was poured dropwise into a cold and concentrated solution of ammonium hydroxide. Recrystallization from ethanol–water gave white plates. M.p. 136–138°.

Anal. Subst. 29.42 mg; 31.62 mg of CO₂; 5.23 mg of H₂O.

C₅H₄BrOSN (206.07): Calc. C 29.14, H 1.97.

Found C 29.31, H 1.99.

Metalation of 3,4-dibromothiophene

To a solution of 12.1 g of 3,4-dibromothiophene in 75 ml of dry ether 45 ml of 1.1 *M* butyllithium was added in a slow stream at room temperature. The solution was stirred for eight hours and was then carbonated and worked up as above. White needles from water–ethanol. M.p. 201–203°. Weight 4.4 g. Yield 31 %. Infrared spectrum, Fig. 3.

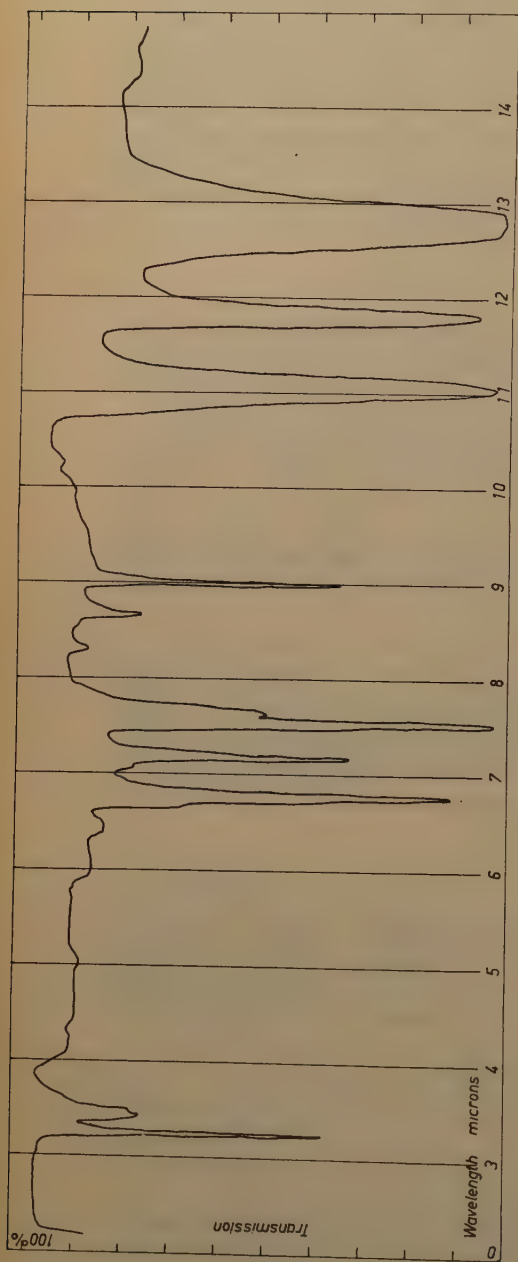
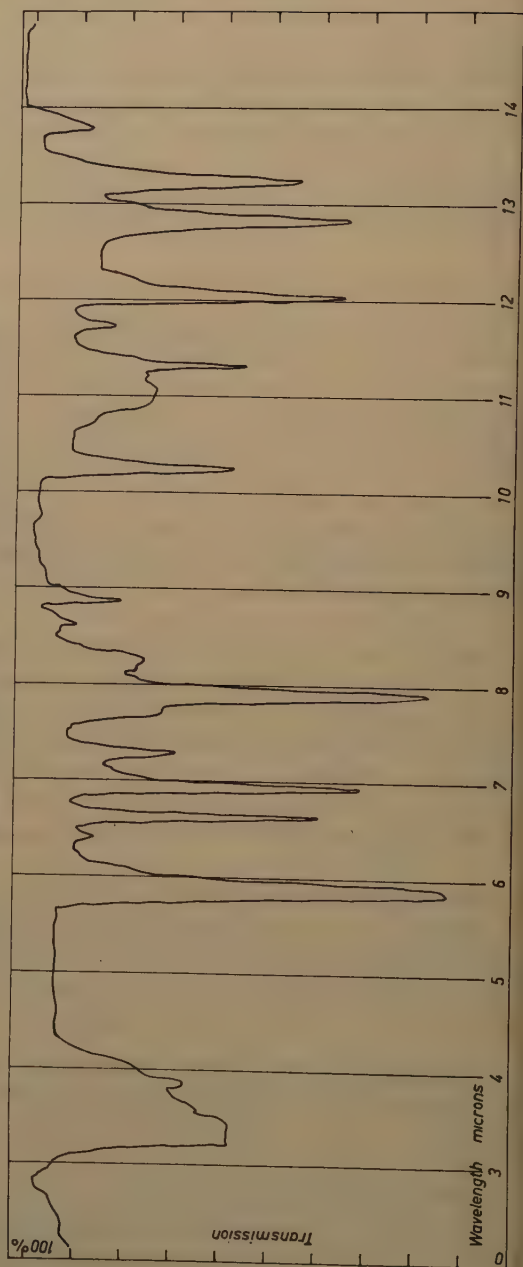


Fig. 1. The infrared spectrum of 3,4-dibromothiophene (liq.).



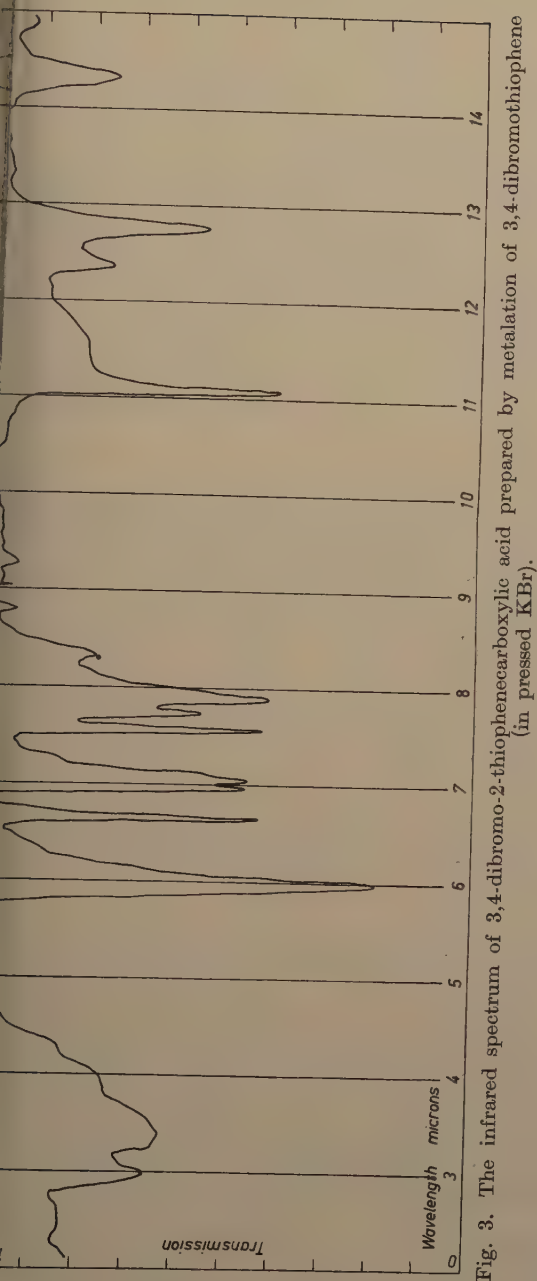


Fig. 3. The infrared spectrum of 3,4-dibromo-2-thiophenecarboxylic acid prepared by metalation of 3,4-dibromothiophene (in pressed KBr).

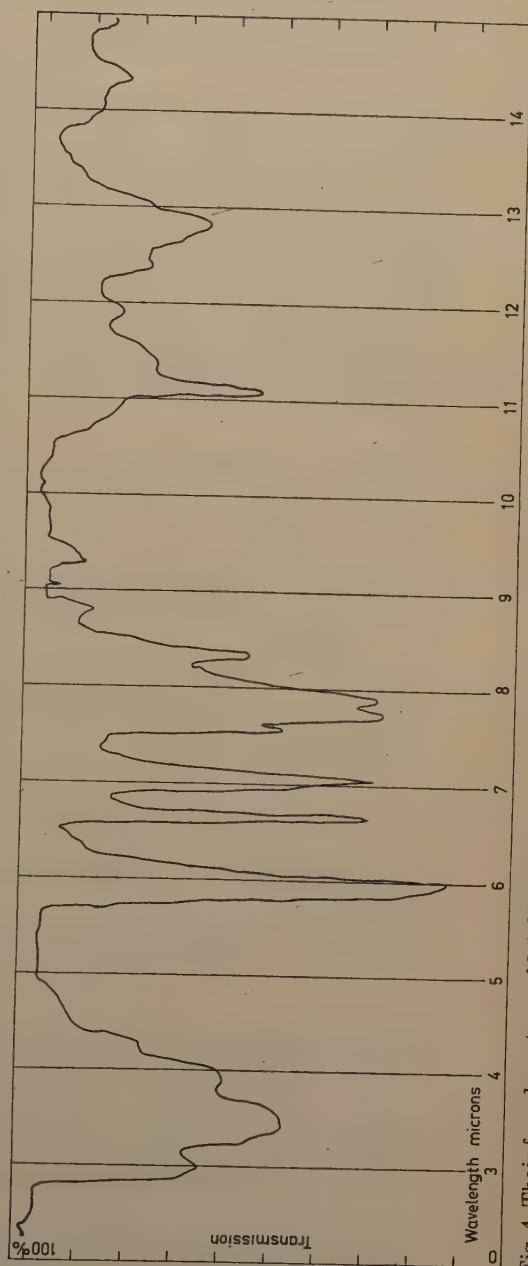


Fig. 4. The infrared spectrum of 3,4-dibromo-2-thiophenecarboxylic acid, prepared from 2,3,4-tribromothiophene (in pressed KBr).

S.-O. LAWESSON, *Thiophene chemistry. Part III*

Anal. Subst. 41.30 mg; 2.81 ml of 0.05106 *M* NaOH.

$C_5H_2Br_2O_2S$: Calc. Equiv. wt. 285.95.

Found Equiv. wt. 287.8.

Preparation of 3,4-dibromo-2-thiophenecarboxylic acid

To a solution of 10 g of 2,3,4-tribromothiophene (19) in 75 ml of dry ether at $-20^{\circ}C$ 38 ml of 0.9 *M* butyllithium (in ether) was added. The solution was then stirred for four minutes, poured on to Dry Ice and worked up in the usual way. Recrystallization from water-ethanol gave white needles. M.p. 201–203°. Weight 5.5 g. Yield 62%. Infrared spectrum, Fig. 4.

Anal. Subst. 37.71 mg; 2.57 ml of 0.05106 *M* NaOH.

$C_5H_2Br_2O_2S$: Calc. Equiv. wt. 285.95.

Found Equiv. wt. 287.3.

4,5-dibromo-3-thiophenecarboxylic acid (II)

To a solution of 2 g of I in 30 ml of glacial acetic acid 1.5 g of bromine in 10 ml of glacial acetic acid was added. The mixture was stirred for 18 hours at room temperature and then poured into ice-water. The precipitate was filtered and recrystallized from ethanol-water, whereby white needles were obtained. M.p. 192–194°. Weight 2.0 g. Yield 72%.

Anal. Subst. 49.30 mg; 3.38 ml of 0.05106 *M* NaOH.

18.52 mg; 14.13 mg of CO_2 ; 1.24 mg of H_2O .

$C_5H_2Br_2O_2S$: Calc. Equiv. wt. 285.95, C 21.00, H 0.71.

Found Equiv. wt. 285.7, C 20.81, H 0.75.

4,5-Dibromo-3-thiophenecarboxylic amide (V)

The preparation as above. White plates from ethanol-water. M.p. 166–168°.

Anal. Subst. 35.25 mg; 27.00 mg of CO_2 ; 3.29 mg of H_2O .

$C_5H_3Br_2OSN$ (284.98): Calc. C 21.07, H 1.06.

Found C 20.9, H 1.04.

2,4,5-Tribromo-3-thiophenecarboxylic acid (IV)

To 2 g of II in 25 ml of glacial acetic acid 4 g of bromine was added. The mixture was refluxed and then poured into water. After decolourizing with bisulfite, the usual procedure was followed. Recrystallization from ethanol-water gave white plates. M.p. 202–204°. Weight 1.98 g. Yield 77%.

Anal. Subst. 71.42 mg; 3.82 ml of 0.05106 *M* NaOH.

$C_5HBr_3O_2S$: Calc. Equiv. wt. 364.9

Found Equiv. wt. 366.1.

IV was also prepared by treating I with bromine.

2,4,5-Tribromo-3-thiophenecarboxylic amide. (VII)

Prepared as above. White plates from water-ethanol. M.p. 219–221°.

Anal. Subst. 31.51; 19.21 mg of CO₂; 1.88 mg of H₂O.

C₂H₂Br₃OSN (363.89): Calc. C 16.50, H 0.55.

Found C 16.62, H 0.67.

4-Bromo-5-nitro-3-thiophenecarboxylic acid (VI)

To a solution of 10 ml of concentrated nitric acid, d. 1.41, and 10 ml of concentrated sulphuric acid, d. 1.84, previously cooled to –10° 4 g of I was added in small portions with stirring. After all the acid had been added, the solution was stirred for ten minutes. The nitrating mixture was then poured into ice water to precipitate the acid. The working-up was as above and recrystallization from ethanol-water gave yellow needles. M.p. 236–238°C. Weight 2.8 g. Yield 57 %.

Anal. Subst. 48.01 mg; 3.73 ml of 0.05106 *M* NaOH.

66.69 mg; 58.83 mg of CO₂; 5.06 mg of H₂O.

C₅H₂BrO₄SN (252.06):

Calc. Equiv. wt. 252.1, C 23.82, H 0.80.

Found Equiv. wt. 252.1, C 24.05, H 0.85.

Attempts were made to introduce two nitro groups into I. Even if the temperature was as high as 100°C during one hour only VI was isolated.

4-Bromo-5-nitro-3-thiophenecarboxylic amide (VI)

Yellow plates from water-ethanol. M.p. 188–190°C.

Anal. Subst. 27.43 mg; 24.18 mg of CO₂; 2.82 mg of H₂O.

C₅H₃BrO₃SN₂ (251.07): Calc. C 23.92, H 1.20.

Found C 24.04, H 1.15.

SUMMARY

The preparation of 3,4-dibromothiophene has been performed in a new way by dehalogenation with butyllithium.

The mono-lithium reagent of 3,4-dibromothiophene has been shown to be very reactive. A two-stage mechanism for metalation of 3,4-dibromothiophene at room temperature is proposed.

Bromination and nitration of 4-bromo-3-thiophenecarboxylic acid have been investigated.

ACKNOWLEDGEMENTS

To Professor A. Fredga I wish to express my sincere gratitude for his interest and for valuable discussions.

Thanks are also due to Dr. A. Rosenberg and Mr. S. Bergwall for taking the infrared absorption curves.

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Department of Organic Chemistry, Chemical Institute, University of Uppsala, February 1957.

REFERENCES

1. STEINKOPF, W., JACOB, H., and PENZ, H., *Ann.* 512 (1934), 136.
2. LAWESSON, S.-O., *Arkiv Kemi* (1957), part V, 11, 345.
3. ZIEGLER, K., and TIEMANN, P., *Ber.* 55 (1922), 3406.
4. GILMAN, H., BEABER, N. J., and JONES, H. L., *Rec. Trav. chim.* 48 (1929), 597.
5. VON BRAUN, J., IRMISCH, G., and NELLES, J., *Ber.* 66 (1933), 1471.
6. SALKIND, J., *Ber.* 67 (1934), 1031.
7. VOTOČEK, E., and KÖHLER, J., *Ber.* 47 (1914), 1219.
8. BRUHAT, G., and THOMAS, V., *Compt. rend.* 183 (1926), 297.
9. STEINKOPF, W., PETERSDORF, H.-J., and GORDING, R., *Ann.* 527 (1937), 272.
10. LAWESSON, S.-O., *Acta Chem. Scand.* 10 (1956), 1020.
11. LAWESSON, S.-O., *Arkiv Kemi* (1957), part IV, 11, 337.
12. ROGERS, M. T., and YOUNG, A., *J. Am. Chem. Soc.* 68 (1946), 2748.
13. EVANS, W. V., and PEARSON, R., *ibid.* 64 (1942), 2865.
14. MORTON, A. A., *ibid.* 69 (1947), 969.
15. ROBERTS, J. D., and CURTIN, D. Y., *ibid.* 68 (1946), 1658.
16. SUNTHANKAR, S. V., and GILMAN, H., *J. Org. Chem.* 16 (1951), 8.
17. MORTON, A. A., and CLUFF, E. F., *J. Am. Chem. Soc.* 74 (1952), 4056.
18. GILMAN, H., and MORTON, JR., J. W., in *Adams, Organic Reactions*, Vol. 8. Wiley, New York, 1954.
19. LAWESSON, S.-O., *Arkiv Kemi* (1957), part VI (to be published).
20. BRYCE-SMITH, D., *J. Chem. Soc.* 1956, 1603.
21. KHARASCH, M. S., and REINMUTH, O., *J. Chem. Ed.* 5 (1928), 404.
22. KHARASCH, M. S., and REINMUTH, O., *ibid.* 8 (1931), 1703.
23. KHARASCH, M. S., REINMUTH, O., and MAYO, F. R., *ibid.* 11 (1934), 82.
24. KHARASCH, M. S., REINMUTH, O., and MAYO, F. R., *ibid.* 13 (1936), 7.
25. JONES, R. G., and GILMAN, H., in *Adams, Organic Reactions*, Vol. 6. Wiley, New York, 1951.
26. WITTIG, G., and FUHRMANN, G., *Ber.* 73 (1940), 1197.
27. GILMAN, H., LANGHAM, W., and WILLIS, H. B., *J. Am. Chem. Soc.* 62 (1940), 346.
28. GILMAN, H., LANGHAM, W., and JACOBY, A. L., *ibid.* 61 (1939), 106.
29. GILMAN, H., MOORE, F. W., and BAINE, P., *ibid.* 63 (1941), 2479.
30. WITTIG, G., *Angew. Chem.* 66 (1954), 10.
31. RIED, W., and BENDER, H., *Ber.* 89 (1956), 1574.
32. STEINKOPF, W., and BAUERMEISTER, M., *Ann.* 403 (1914), 50.

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Uppsala 1957. Almqvist & Wiksells Boktryckeri AB

Thiophene Chemistry. Part IV

The preparation of bromosubstituted thiophene Grignard reagents by "entrainment" and transmetalation. An indication of the validity of the Urion exchange mechanism

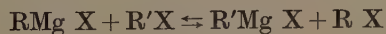
By SVEN-OLOV LAWESSON

Lithium and Grignard reagents are similar in many respects and since the lithium compounds were prepared by Ziegler and coworkers (1) in 1930 and shown to be usable in place of the corresponding magnesium compounds intensive research has been performed in this field. It has been shown that lithium reagents in many cases are superior to those of magnesium but that owing to the high reactivity of the former, side reactions may easily occur and must be avoided by working under suitable and in fact special conditions. In thiophene chemistry this is sometimes a complication and therefore we have investigated the preparation of Grignard reagents from bromothiophenes by different methods.

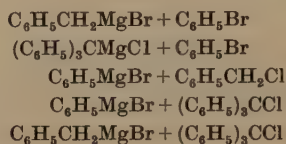
The preparation of Grignard reagents from 2-bromo- and 2-iodothiophenes is a very smooth reaction. The induction period is very short and in reactivity these compounds can be compared to alkyl halides. However, if we started from a thiophene derivative with more than one substituent it was impossible, at least in most cases, to form the Grignard reagent in the common way. Attempts to initiate the reaction with the usual activators such as iodine or small amounts of ethylmagnesium bromide failed. The bromine activator, first introduced by Taboury (2), which has been found here in Uppsala to initiate halogen-magnesium reactions in sterically hindered polycyclic and polyheterocyclic compounds, gave no results.

In 1934, Steinkopf (3) and Grignard (4), independently of each other, introduced the method of "entrainment", which means that one mixes the sluggish-reacting component with a very reactive alkyl halide, often ethyl bromide, and then continues according to the general procedure. Steinkopf *et al.* prepared in that way a great many thiophene derivatives (5, 6, 7, 8, 9) but they made no investigation of the mechanism or the variation of the yield with different amounts of the auxiliary halide. Often they did not mention anything about such important matters as yields and procedure.

In France, however, Grignard himself (5, 10) and his coworkers Urion (11) and Clement (12, 13) made more extensive experiments. As to the mechanism, Urion was of the opinion that there was a halogen-magnesium exchange reaction according to:

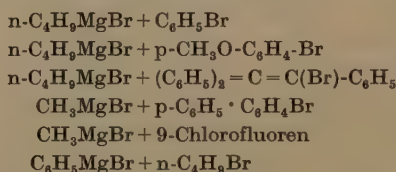


That is, the Grignard reagent easily formed from the auxiliary halide, will in the above equilibrium react with the non-reacting halide to form its Grignard compound. Such interconversion reactions are known from lithium-halogen exchange reactions, but the example with which Urion tried to show the validity of his theory for the Grignard reagent, was not conclusive (14). Five years earlier, Gilman and Jones (15) had tried to detect some exchange between a Grignard reagent and a halide but obtained no reaction:



Clement studied the variation of the yields with the amount "entrainer" and concluded that at least one mol should be used per mol of non-reacting halide. He found, too, that methyl bromide gave the best yields, that no difference existed between different halides, RX ($\text{X} = \text{Cl}, \text{Br}$) and that allyl halides could be used as entraining agents.

Later on, Kharasch and Fuchs (14) found that the following Grignard reagents and organic halides did not react at a temperature of about 0°C :



This confirmed the results of Gilman and Jones and appeared to rule out the theory of Urion.

In attempts to prepare the mono-Grignard reagent of the dibromothiophene derivatives without using any other activator than bromine it was found that only 2,5-dibromothiophene reacted. In spite of prolonged heating the yield could not be increased above 15-20 per cent. The reason seems to be that the Grignard reagent is insoluble in ether and thus the magnesium becomes coated with a surface layer which hinders the reaction. However, by using ethyl bromide as entraining agent the yield could be increased and we could observe in this case that the yield increased with mol entrainer per mol reactant. The yield was determined by carboxylation and isolation of the acid. This method is in no way satisfactory because side reactions, leading to the formation of a ketone or a tertiary alcohol, always reduce the yield of the acid. Throughout this investigation we have added the cooled Grignard reagent solution to excess of an ethereal suspension of solid carbon dioxide thus attempting to arrest the reaction at the carboxylate stage. The yield of acid is not equal to that of the corresponding Grignard reagent but it is a relative measure of it.

From the tribromo- and tetrabromothiophenes the corresponding acids were prepared under the same conditions as above. The preparation of Grignard reagents was impossible without entraining agent but by using 5 mol of an auxiliary halide per mol reactant good yields were obtained:

Table 1. Preparation of bromo-substituted thiophene-carboxylic acids by "entrainment".

Reactant	Time (hour)	Yield (%)
2,3-dibromothiophene ^a	20	41
2,4-dibromothiophene ^b	20	51
2,5-dibromothiophene ^a	15	60
3,4-dibromothiophene ^c	19	38
2,3,4-tribromothiophene ^a	19	54
2,3,4,5-tetrabromothiophene ^a	20	50

^a Prepared according to Lawesson (16).^b Prepared according to Lawesson (17).^c Prepared according to Lawesson (18).

From Table 1 we see, that in order to have good yields the reaction time must be prolonged. Often, however, the yields were smaller depending on the fact, that the crude products were impure and much was lost in the work-up. The exchange reaction, proposed by Urion, seems to be partly supported by these experiments. For if there exists an equilibrium, and this is by no means improbable, it must be displaced to the right if the concentration of the alkyl-magnesium halide is increased. In all our experiments here and in earlier papers in this series we have found that the yields were higher if the ratio of entraining agent to thiophene halide increased. With reference to the bromothiophenes this in turn means that the equilibrium is favourably displaced to the right and for this reason the corresponding derivative of a thiophene-carboxylic acid is isolated in a good yield. We observed, too, that the smallest yield was obtained when the $-MgBr$ group was in a β -position, thus indicating that the sulfur may have some influence.

In order to investigate if an interconversion really occurred ethylmagnesium bromide was refluxed with 2-bromothiophene for 4 hours. After carboxylation, the thiophene acid was isolated and characterized. An exchange reaction undoubtedly occurred and the yields have been shown to increase with the ratio of ethylmagnesium bromide to thiophene halide. From Table 2 this is obvious.

Table 2. Interconversion reactions between 2-bromothiophene and ethylmagnesium bromide.

2-Bromothiophene (mol)	Ethylmagnesium bromide (mol)	Yields ^a of thio- phenemagnesium bromide (%)
0.1	0.1	11
0.1	0.3	49
0.1	0.5	64

^a Based on the carboxylic acid.

If we used 3-bromothiophene or 3,4-dibromothiophene instead of 2-bromothiophene no interconversion reaction could be observed. However, if the ether was evaporated and a small amount of dry benzene was added, some exchange occurred. Table 2 shows the results from 10 experiments with various halothiophenes when 5 mol of ethylmagnesium bromide was used per mol reactant.

Table 3. Interconversion reactions between halo-substituted thiophenes and ethylmagnesium bromide.

Reactant	Time (hour)	Yield ^a %
2-bromo-thiophene	4	64
2-iodo-thiophene ^c	2	76
3-bromo-thiophene ^d	20 ^b	10
2,3-dibromothiophene	4	21
2,4-dibromothiophene	4	29
2,5-dibromothiophene	4	73
3,4-dibromothiophene	20 ^b	7
2,3,4-tribromothiophene	4	54
2,3,4,5-tetrabromothiophene	4	75
2,3,4,5-tetraiodothiophene ^e	24	4

^a Based on the carboxylic acid formed.

^b Refluxed in benzene.

^c Prepared according to Minnis (19).

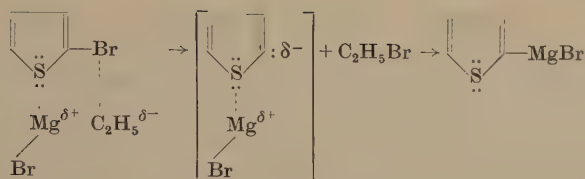
^d Prepared according to Lawesson (16, 20).

^e Prepared according to Steinkopf *et al.* (8).

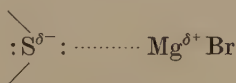
From the experiments, listed in Table 3, the acids were easily isolated and no difficulties arose with the work-up. The reaction time was short, except when "forced" conditions were used in order to introduce a $-\text{MgBr}$ group into a β -position, and no polymers or similar substances were formed. As to 2,3,4,5-tetraiodothiophene, this compound is almost insoluble in ether and this is probably the cause of the low yield.

In Table 3 it is seen that the $-\text{MgBr}$ group was easily introduced into an α -position. The interconversion reaction with a halogen ortho to the sulfur in the polyhalogenthiophenes was much more rapid than the interconversion with halogen atoms in other positions. The reactivity of halogens increased as the electronegativity of the element decreased, i. e. the reactivity series $\text{J} > \text{Br}$. No more than one halogen was changed, i. e. only the mono- MgBr compound was formed irrespectively of the amount of ethylmagnesium bromide.

It is assumed that because the reactivity of the halogens depended upon the relative electronegativity of the element, the anion of the Grignard reagent, by a nucleophilic mechanism, attacks the halogen, which is removed in its positive condition. Furthermore, as the α -halogen was always removed, an intermediate coordination step is proposed according to:



It is known since the time of Grignard, that it is almost impossible to prepare a Grignard reagent in a reaction medium which is not an electron donor (21). The ether, for instance, coordinates with magnesium to compensate for the polarity of the metal. This ether-metal coordination is of great importance as a hindrance to coupling etc. Furthermore, if reaction occurs, all substances coordinate with the Grignard reagent (22). Thus here we have an exchange between the ether and the reactant, and a reaction will not take place if the reactant is weakly electron-donating compared to the ether. In the examples above the coordination of the ethylmagnesium bromide etherate compound to the free electron pair of the sulfur will displace one or more of the highly polarized solvent molecules. The case of exchange will depend upon relative electron donating power. By this the solvent molecules will not hinder the $\text{C}_2\text{H}_5^{\delta-}$ from attacking the bromine. It is possible, too, that the bond



will be stronger, if the sulfur is a part of a relatively highly polarizable aromatic system, than the bond



when the oxygen is part of a saturated system; e.g. ether. This will have the effect of decreasing the attraction between $\text{Mg}^{\delta+} \text{Br}$ and $\text{C}_2\text{H}_5^{\delta-}$, making the latter more available for attack on the halogen.

Because of the difficulty to introduce the $-\text{MgBr}$ group into the β -position of 3-bromothiophene or 3,4-dibromothiophene, it is obvious that any sulfur-magnesium coordination cannot facilitate the reaction in ether. Instead another mechanism must exist. It seems to be clear, however, that the bromine is weakly electron-donating compared to ether. If we replaced the ether with benzene and performed the experiments at higher temperature and for a longer time we could bring about exchange. The higher temperature must be an advantage but the chief benefit comes from the fact, that the heat of solvation of Grignard reagents in hydrocarbons is small in comparison to that in ethers.

From the results above one may conclude that in thiophene chemistry, halogen-metal interconversions exist in the case of Grignard reagents. The reaction equilibrium in the transmetalation is favourably displaced and perhaps facilitated by the coordination to the hetero-atom. It must, however, be emphasized that the results from Table 3 may be an indication only. In the experiments of Kharasch and Fuchs (14) no interconversion reaction could be detected

but if cobaltous chloride was added, small amounts of exchange products were isolated as carboxylic acids. It is not improbable that the method of "entrainment" implies partly a free-radical mechanism, induced by traces of magnesium-halides or other radical-forming reactant.

The mechanism involved in the preparation of Grignard reagents by "entrainment" is probably very complex and no general theory can be put forward at present. It is typical that magnesium compounds of organic halides which do not yield the Grignard reagents under ordinary conditions are very insoluble in ether. The magnesium compound of the entraining agent may perhaps function only as a solvating agent in many cases, thus making the Grignard reagent of the sluggish-reacting halide more soluble and in that way cleaning the surface of the magnesium. The formation of the Grignard reagent does not cease and the corrosion of the magnesium surface is not hindered by a coating on it.

Experimental

All preparations and reactions of Grignard reagents were carried out in a common Grignard apparatus and in nitrogen atmosphere. Commercial magnesium turnings were used.

The experiments listed in Tables 1 and 2 need no comments as to the procedure, which has been described in earlier papers in this series (17, 18).

The experiments listed in Table 3 were conducted as follows: 75 ml of absolute ether containing 0.1 mol of ethylmagnesium bromide was filtered free from magnesium. To this solution 0.02 mol of the reactant was added and the whole refluxed for four hours. After the mixture had cooled it was then treated with an excess of carbon dioxide in dry ether. The work up of the product was performed in the usual manner.

3,4,5-triiodo-2-thiophenecarboxylic acid

Yellow plates from ethanol-water. M.p. 240–243 (d).

Anal. Subst. 85.41 mg; 3.28 ml of 0.05106 *M* NaOH.

27.48 mg; 12.11 mg of CO₂.

C₅HJ₃SO₂: Calc. Equiv. wt. 505.85; C 11.89.

Found Equiv. wt. 510.1; C 12.01.

SUMMARY

The preparation of Grignard reagents from polybromosubstituted thiophenes has been shown to be impossible under the usual conditions without the use of an entraining agent.

Interconversion reactions with Grignard reagents have been shown to exist in the case of bromosubstituted thiophenes. The introduction of the MgBr group into the α -position is a smooth reaction. A similar reaction occurs at the β -position under "forced" conditions. A mechanism is proposed.

A new acid, 3,4,5-triiodo-2-thiophenecarboxylic acid, has been prepared.

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To Professor Arne Fredga I wish to express my sincere gratitude for his interest in this work and for many valuable discussions,

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Department of Organic Chemistry, Chemical Institute, University of Uppsala, February 1957.

REFERENCES

1. ZIEGLER, K., and COLONIUS, H., *Ann.* 479 (1930) 135.
2. TABOURY, F., *Ann. chim.* [8], 15 (1908), 5.
3. STEINKOFF, W., JACOB, H., and PENZ, H., *Ann.* 512 (1934), 136.
4. GRIGNARD, V., *Compt. rend.* 198 (1934), 625.
5. STEINKOFF, W., *Ann.* 513 (1934), 281.
6. STEINKOFF, W., and JACOB, H., *ibid.* 515 (1935), 273.
7. STEINKOFF, W., SCHMITT, H. F., and FIEDLER, H., *ibid.* 527 (1937), 237.
8. STEINKOFF, W., and HANSKE, W., *ibid.* 527 (1937), 264.
9. STEINKOFF, W., and HANSKE, W., *ibid.* 532 (1937), 236.
10. GRIGNARD, V., *Compt. rend.* 198 (1934), 2217.
11. URION, E., *ibid.* 198 (1934), 1244.
12. CLEMENT, H., *ibid.* 198 (1934), 665.
13. CLEMENT, H., *Ann. chim.* [11], 13 (1940), 243.
14. KHARASCH, M. S., and FUCHS, C. F., *J. Org. Chem.* 10 (1945), 292.
15. GILMAN, H., and JONES, H. L., *J. Am. Chem. Soc.* 51 (1929), 2840.
16. LAWESSON, S.-O., *Arkiv Kemi* (1957): Part VI, to be published.
17. LAWESSON, S.-O., *ibid.* (1957): Part II, 11, 317.
18. LAWESSON, S.-O., *ibid.* (1957): Part III, 11, 325.
19. MINNIS, W., *Organic Syntheses* 12 (1932), 44.
20. LAWESSON, S.-O., *Acta Chem. Scand.* 10 (1956), 1020.
21. GRIGNARD, V., *Ann. chim.* [7] 24 (1901), 433.
22. STRAUS, F., *Ann.* 393 (1912), 235.

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Uppsala 1957. Almqvist & Wiksells Boktryckeri AB

Thiophene Chemistry. Part V

Dehalogenation of bromosubstituted thiophenecarboxylic acids with butyllithium. An indication of a nucleophilic mechanism in the bromine-lithium interconversion

BY SVEN-OLAV LAWESSON

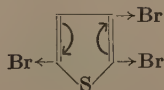
For synthetic work, halogen-lithium interconversion is preferable to lithium metalation. The former can be performed at a very low temperature, the exchange is momentary and the substitution can be made in a position which can generally be predicted. Metalation, on the other hand, is a more time-consuming reaction and the conditions are not so mild.

The mechanism for lithium-halogen exchange and metalation is claimed to be similar. However, there are two interpretations, the electrophilic one (1) which seems to go to extremes and the nucleophilic one (2, 3), which is nowadays regarded as plausible. Indications of a nucleophilic mechanism have been shown to exist by us (4, 5).

Nucleophilic substitution reactions have not interested organic chemists as much as electrophilic substitution e.g. halogenation, nitration, sulfonation and alkylation. Organic lithium chemistry has now become an indispensable tool for the synthetic chemist and we have found it interesting to try to solve the problem of whether an electrophilic or nucleophilic mechanism is involved in the lithium-halogen interconversion.

In general one cannot speak about directing effects in nucleophilic substitutions, for often there is only one replaceable group which if activated will be replaced and if not, no reaction will occur. In aromatic polyhaloderivatives we have more than one replaceable group and the opportunity is thereby presented to investigate the directing effects of different functional groups.

In the dehalogenation of 2,3,5-tribromothiophene with butyllithium at low temperature (5,6), the lithium entered into the 2-position which indicated a typical nucleophilic attack. The bromine is an ortho-para directing group in electrophilic substitution, where the tautomeric and inductive effects are oppositely directed but where the tautomeric effect dominates.



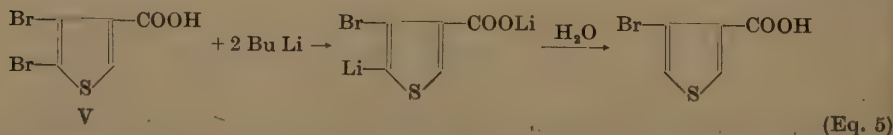
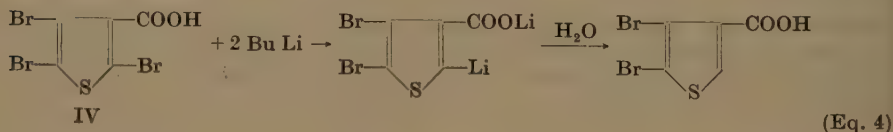
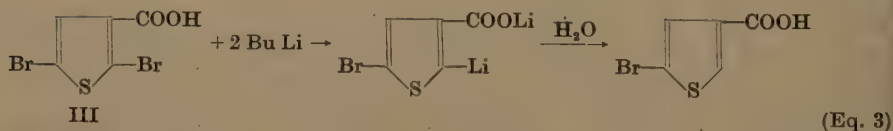
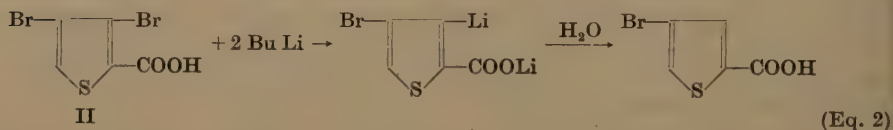
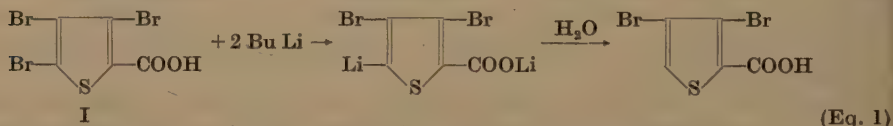
In nucleophilic substitution the inductive effect ($-I$) generally plays the most important role. Furthermore, with respect to halogen-lithium interconversion

reactions, no studies have hitherto been made of the influence of such groups as $-\text{COOH}$, $-\text{CN}$, $-\text{COR}$ and $-\text{COOR}$, which in electrophilic substitution are typically meta-directing. Generally such a study is impossible in metalating experiments, which must occur at least at the reflux temperature of ether, because reaction of the lithium reagent with these groups occurs faster than nuclear metalation.

In lithium-bromine interconversions the carboxylic group is very suitable if the temperature is low (-70°C), because no addition to the carbonyl group occurs, but only the lithium salt is formed. By treating a polyhalosubstituted aromatic carboxylic acid with 2 moles of butyllithium at a low temperature, one mol of the reagent is used for the formation of the lithium salt and the other for bromine-lithium interconversion. By hydrolyzing we obtain a new acid which can be characterized, and thus the point of attack can be determined.

A disadvantage of metalation is that the reaction must often proceed for 10–20 hours and thus producing a number of equilibrium systems. It is thus not possible to predict the direction in which the equilibrium will shift. For instance, as was shown in earlier papers in this series (4, 5) side reactions predominate at room temperature and the only way to avoid these is to perform the experiment at a very low temperature, whereby only the normal and expected products are isolated. The reaction time must be short, too, and generally is not longer than a few minutes.

The following reactions have been performed:



We know that in the thiophene kernel the two α -positions are activated by the sulfur for both electrophilic and nucleophilic substitution (7). From eq. 1 it is clear that the bromine in the 5-position is attacked, and this stresses the fact that the sulfur in thiophene mainly controls the substitution.

To interpret eq. 2, 3 and 4 is more difficult. According to the symbolism of Ingold (8) an aromatic carboxylic acid is of the type $-I-T$ and the ionized carboxylic salt of the type $+I-T$. In all cases above, the first mol of butyllithium will form the lithium salt, so it is not the directing effect of the carboxylic group but the carboxylate that has been investigated here.

In a carboxylic acid both the inductive and tautomeric effects are in the same direction, e.g. electrons are pulled from the aromatic part and thus the meta-positions will be least positive. If, as in these cases, we have a carboxylate there are two conflicting effects. The negative ion functions as an electron-repelling group while the tautomeric effect acts in the opposite direction. However, even in this case the tautomeric effect predominates and the carboxylate is chiefly meta-directing in electrophilic substitution.

All experiments were performed in ether solution. The ether has a low dielectric constant and the lithium salt will form an emulsion which presumably is very slightly ionized. The inductive effect of the salt in ether is believed to be very small compared to the tautomeric effect and the latter is wholly predominant. In fact, in rough outline, the carboxylic group and an almost unionized carboxylate will behave similarly and have the same influence in substitution reactions. From that it is clear, that if the halogen-lithium interconversion reaction occurs according to a nucleophilic mechanism, the ortho- and para-positions to the carboxylate will be attacked. This is also confirmed by eq. 2, 3 and 4. In eq. 5 we see that the meta position was attacked. This may be understood from the fact that it is an α -position to a sulfur hetero atom and further stresses the influence of the hetero atom.

Experimental

All reactions were carried out in nitrogen atmosphere and in the usual type of apparatus.

Preparation of 3,4,5-tribromo-2-thiophenecarboxylic acid

To a solution of 20 g of 2,3,4,5-tetrabromothiophene (9) in 125 ml of absolute ether at -20°C 42 ml of 1.2 *M* butyllithium (in ether) was added. The solution was stirred for 10 minutes and was then poured on to Dry Ice. After the Dry Ice had evaporated the mixture was hydrolyzed with dilute acid, the organic layer separated, the water solution extracted with ether and the combined ether layers extracted with dilute sodium hydroxide. Upon acidification of the alkaline solution a white powder precipitated; this after recrystallisation from formic acid gave white plates. M.p. 258–261. Weight 13.3 g. Yield 73 %.

Debrominations

In Table 1 the results of 5 debromination experiments are given. The procedure is simple and the debromination of 3,4,5-tribromo-2-thiophenecarboxylic acid may be taken as a typical example.

Debromination of 3,4,5-tribromo-2-thiophenecarboxylic acid.

To a solution of 3.65 g (0.01 mol) of 3,4,5-tribromo-2-thiophenecarboxylic acid in 30 ml of absolute ether at -70°C 10 ml of 1 *M* butyllithium was added very quickly. The stirring was continued for 3 minutes and the mixture was then poured into dilute acid. The organic layer was separated, the water phase shaken with ether and the combined ether layers extracted with dilute sodium hydroxide. Upon acidification of the alkaline solution a white powder precipitated; this after recrystallisation from water-ethanol gave fine plates. M.p. $201-203^{\circ}\text{C}$. Weight 1.63 g. Yield 57 %.

Anal. Subst. 31.29 mg; 2.14 ml of 0.05106 *M* NaOH.

$\text{C}_5\text{H}_2\text{Br}_2\text{O}_2\text{S}$: Calc. Equiv. wt. 285.95.

Found Equiv. wt. 287.1.

Table. 1. Debromination of bromosubstituted thiophenecarboxylic acids.

Reactant	Product	M.p. (°C)	Equiv. wt.	Yield (%)
I	3,4-dibromo-2-thiophenecarboxylic acid	201-203	287.1	57
II ^a	4-bromo-2- "	122-124	208.4	50
III ^b	5-bromo-3- "	117-119	207.9	49
IV ^a	4,5-dibromo-3- "	192-194	286.8	47
V ^a	4-bromo-3- "	157-159	207.2	53

^a Prepared according to Lawesson (4).

^b Prepared according to Campaigne *et al.* (10).

SUMMARY

Polybromothiophenecarboxylic acids have been treated with two moles of butyllithium at -70°C and after hydrolysis the corresponding acids having one bromine atom replaced by hydrogen have been obtained. The results indicate a nucleophilic mechanism.

ACKNOWLEDGEMENTS

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Department of Organic Chemistry, Chemical Institute, University of Uppsala, February 1957.

REFERENCES

1. MORTON, A. A., *J. Am. Chem. Soc.* **69** (1947), 969.
2. ROBERTS, J. D., and CURTIN, D. Y., *ibid.* **68** (1946), 1658.
3. SUNTHANKAR, S. V., and GILMAN, H., *J. Org. Chem.* **16** (1951), 8.
4. LAWESSON, S.-O., *Arkiv Kemi* (1957): Part III, **11**, 325.
5. LAWESSON, S.-O., *ibid.* (1957): Part II, **11**, 317.
6. LAWESSON, S.-O., *Acta Chem. Scand.* **10** (1956), 1020.
7. DE HEER, J., *J. Am. Chem. Soc.* **76** (1954), 4802.
8. INGOLD, C. K., *Structure and Mechanism in Organic Chemistry*. Cornell University Press, Cornell University Press, Ithaca, N.Y., 1953.
9. LAWESSON, S.-O., *Arkiv Kemi* (1957): Part VI, to be published.
10. CAMPAIGNE, E., and BOURGEOIS, R. C., *J. Am. Chem. Soc.* **76** (1954), 2445.

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Rapid radiochemical separations of polonium isotopes from wolfram targets bombarded by cyclotron-accelerated neon ions

By GERDA OELSNER and WILHELM FORSLING

With 3 figures in the text

ABSTRACT

The radiochemical separation of short-lived polonium isotopes, produced by the nuclear reactions of cyclotron-accelerated neon ions in metallic wolfram targets is studied.

Two of the methods investigated are described in detail. A simple technique for volatilization direct from the target has been developed, in which the target surface is dissolved before the evaporation.

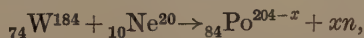
Also, conditions for rapid electrolytic separation have been established. The quick dissolution of the target, and the quantitative influence of hydrofluoric acid and nitric acid concentrations on the electrodeposition of polonium have been investigated. The recommended electrolytic solutions are 0.2–0.5 *N* in HF, 0.4–0.7 *N* in HNO₃ and 0.1 % in tartaric acid, for a concentration of 50 millimoles wolfram per litre.

Lastly, a discussion on the estimation of the maximum obtainable final yield of short-lived polonium isotope samples by electrolysis under the present conditions, is given.

Introduction

Studies of the species formed by the nuclear reactions of cyclotron-accelerated neon ions with various target materials have been made at this institute. One of the radiochemical problems arising when metallic wolfram foils were used as targets (1), was the separation of short-lived polonium isotopes.

The nuclear reactions of the neon ions in the wolfram targets leading to polonium are of the type:



where n symbolizes a neutron and x is the number of neutrons, generally 4–6, released from the compound nucleus.

Various methods were investigated in an attempt to overcome the chemical difficulties involved by the presence of wolfram and the hydrofluoric acid necessary to dissolve the wolfram target. Any chemical manipulation which was tested, in an

attempt to remove the hydrofluoric acid, caused the wolfram to be precipitated as hydrated oxide, carrying with it some of the polonium.

The methods tried for separating polonium may be summarised as follows.

(a) Solvent extraction using tributyl phosphate (T.B.P.) in dibutyl ether (2). This was found to be inapplicable in our case because of the presence of hydrofluoric acid.

(b) Coprecipitation on tellurium. Polonium can be precipitated with a tellurium carrier which is reduced to the metal by stannous chloride (3). However, trials with this method were unsuccessful because of the precipitation of the wolfram oxide. Even if this difficulty were avoided, the method would be too time-consuming to be useful for the separation of short-lived isotopes.

(c) The recoil method. In this physical separation, the target foil is so thin that product nuclei from the ion bombardment are ejected from the target and collected in catcher-foils placed behind it. This method has been used by several investigators (e.g. 4, 5) and was also applied to the separation of polonium from the wolfram targets. The chemical problem was here the preparation of the necessary thin wolfram films. The technique used at our laboratory will be described elsewhere (6).

(d) Volatilization from the wolfram target direct onto a cooled metal plate, e.g. platinum. Volatilization of carrier-free tracers is widely used in radiochemistry, both from solutions and from the surface of solid substances. For the purification of polonium, much work has been done along these lines (7). An evaporation direct from the target has apparently not been reported for polonium. Such a volatilization of astatine from a gold target was done by Hollander (8). At this laboratory, heavier actinides, e.g. californium, were successfully evaporated directly from uranium targets. Many attempts showed that polonium could not be evaporated in this way. However, by first dissolving the target surface with a few drops of acid, the polonium could be volatilized, and the speed of the total separation was still sufficient for the study of short-lived polonium isotopes.

(e) Electroplating seems generally to provide the most satisfactory method for the separation of polonium (9, 10, 11, 12). It appears that the electroplating of polonium from solutions containing hydrofluoric acid, necessary in our case for dissolving the wolfram, has not previously been investigated. Thus, some experiments to study the quantitative trend of the dependence of polonium deposition on acid concentrations, especially with respect to hydrofluoric acid, were carried out.

The volatilization method

The usefulness of volatilization for the radiochemical separation of polonium depends upon the fact that no other volatile compounds are formed in the nuclear reactions or in the succeeding chemical procedure, which could vitiate the final measurements of the polonium radioactivity.

Experimental procedure

The "thick target" foils used in the bombardments were approximately 13×19 mm in size and had a thickness of about 45μ . Immediately after the cyclotron bombardment, the target was placed under an infra-red lamp to be heated. A few drops of a solution, which was about 9 *N* in HF and 2 *N* in HNO₃, were pipetted onto the target surface and spread over the entire bombarded area. In the minute during

which the acid evaporated, dissolution of the wolfram surface occurred, producing a thin salt layer. The dried target was then placed on a grid support. A half-centimeter thick asbestos ring, lying around the wolfram plate, acted as heat insulator and collimator, while a platinum plate, lying on the ring, caught the quickly distilled polonium from the heated target. It was necessary to cool the platinum catcher foil e.g. with a small amount of moist tissue. A similar distillation technique has been used in Berkeley (2).

A good evaporation yield could be obtained if the target was heated to a faint red colour for about 30 seconds. To prepare very thin alpha-radioactive tracer samples, it is recommended that the distillation be repeated.

Discussion of the volatilization method.

As neutron deficient bismuth and polonium nuclides are expected to have rather similar alpha-radioactive properties (13), it was necessary to test whether bismuth, which is likely to be formed during the bombardment, is also evaporated under the conditions described above for polonium. From available data, this does not seem impossible (17). An experiment with Bi^{210} as tracer showed, however, that not more than 1 % of the bismuth activity evaporated, compared with up to 50 % for polonium. Thus, by repeating the evaporation once, it can be tested whether a certain unknown activity is to be referred to bismuth or to polonium.

In order to make a rough evaluation of the yield of polonium from both the dissolution and the evaporation, some experiments with inactive wolfram foils were carried out. Using the procedure described above, and thereby adding 0.2 ml of the HF-HNO_3 acid mixture to the target surface, it was found that a mean thickness of 2.5μ of the metal surface was dissolved. Since the maximum intensity in the energy spectrum of the accelerated neon ions lay at an energy of about 130–140 MeV, and the energy necessary that the $\text{W}(\text{Ne}, xn)\text{Po}$ reactions can take place is about 100 MeV, one can calculate that the effective range of the neon ions within the wolfram target is about 7.5μ . Without taking into consideration the energy distribution of the neon ions or the excitation functions of the reactions (as these experimental data are not available), one can thus deduce that roughly one third of the total polonium atoms formed is found in the dissolved surface layer, and that the combined yield of dissolution and evaporation is somewhat less than 20 %. As the method is very rapid (2–3 minutes), it was of value for studying polonium isotopes having half-lives as short as 2 to 4 minutes (6).

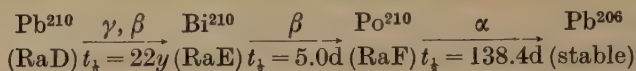
An improvement of the yield could be obtained by deeper dissolution of the target. However, the disadvantages arising here would be that the separation would take longer, that on dissolution a thicker salt layer would be formed which would give greater likelihood of spluttering onto the platinum plate and also hinder somewhat the escape of polonium during the volatilization.

The electrolytic deposition method

Chemical preparations

The polonium tracer.—The Po^{210} used as tracer was separated from old radon ampoules by electroplating from a 0.4 N nitric acid solution onto a silver plate fastened into the end of a rotating glass rod (14). Possible contaminating activities

in the final polonium(IV) solution are Pb^{210} and Bi^{210} , which belong to the decay series:



By measuring a sample of the polonium solution in a gamma-scintillation counter, it was found that no gamma-rays were present, i.e. no Pb^{210} contaminated the solution. However, by measurements with a G.M.-tube, some beta activity was found, indicating the presence of Bi^{210} . This small contamination was shown to have no influence on the electrodeposition yield of the polonium.

To estimate the amount of polonium tracer added to the solutions to be electroplated, the procedure below was followed for each run: from the same sample in a pipette, successive 0.1 ml aliquots of the acidic Po^{210} solution were run out, the first onto a clean platinum plate, the second into the solution, and the last onto another clean platinum plate. The measurement of the 0.1 ml volumes was accurate to 0.5%. The polonium solutions on the platinum plates were dried under a heat lamp and counted for alpha activity in a zinc-sulphide scintillation counter. Tests showed that no polonium loss occurred during the heating under the infra-red lamp. The average of the counts on the two platinum plates was assumed to give the number of alpha-counts in the aliquot of Po^{210} added to the solution. The reproducibility obtained by this sampling method was within 10%. This error could not easily be reduced, but gave sufficient accuracy for the present experiments. As a single polonium sample was used to study the yield-time dependence for a certain acid concentration, the errors in each series of measurements were probably less. This becomes obvious from the relatively small deviations of the points from smooth curves in Figs. 1 and 2.

Wolfram dissolution.—To find the optimum proportions of hydrofluoric and nitric acids, and also the amounts of these acids necessary to just dissolve a certain weight of wolfram target, the following trials were made.

0.184 g (one millimole) of 45 μ -thick wolfram foil (the same as was used for targets in the cyclotron bombardments) was cut into small pieces, treated with various proportions of the acids, and left to stand in a boiling water bath. The following proportions were tested:

HF (10.7 N) in ml	HNO_3 (14.2 N) in ml	Remarks about the wolfram metal
4.5	0.05	did not dissolve in 30 min
4.5	0.40	dissolved in 3 min
2.0	0.40	dissolved in 1.5 min
1.0	0.25	dissolved in 1.5 min
1.0	0.20	dissolved in 2 min
0.8	0.20	dissolved in 3 min
0.75	0.20	some oxide precipitated immediately after dissolution
0.5	0.20	the oxide began to precipitate before dissolution was complete
0.8	0.75	dissolved in 3 min, the oxide precipitated after 15 min at 40°C

Since an excess of HF had to be avoided for the later electrolysis procedure, the optimum mixture of HF- HNO_3 to dissolve 0.184 g W was taken to be 1.0 ml 10.7 N HF and 0.2 ml 14.2 N HNO_3 , which per millimole W is 10.7 millimole HF and 2.8

millimole HNO_3 . A convenient solution volume for these electrolyses was 20 ml, since the polonium should not be diluted more than necessary. However, this volume is required to dilute the acids to the normalities of 0.54 and 0.14 in HF and HNO_3 respectively.

From a number of preliminary electrolysis runs with a diluted solution containing only wolfram, hydrofluoric acid and nitric acid, it was seen that a further complexing agent was necessary to hold the wolfram in solution for runs longer than 5 minutes. Tartaric acid was tested and found satisfactory for this purpose.

To find approximately the amount of tartaric acid necessary under the given conditions, the following four solutions were tested, each containing 0.184 g wolfram dissolved in 1.0 ml 10.7 *N* HF and 0.2 ml 14.2 *N* HNO_3 , and diluted with water. Aliquots of 2.0 ml, 1.5 ml, 1.0 ml and 0.4 ml of a 1 % tartaric acid solution were added, respectively, and the solutions made up to a total volume of 20 ml. After standing in a water bath at 30°C for an hour a slight precipitate of hydrated wolfram oxide appeared in the latter two solutions. For all following electrolysis runs, 2 ml of the 1 % tartaric acid solution were added.

The electrolytic cell

The solutions to be electrolysed were held in a 30 ml glass beaker (no obvious attack on the glass occurred with a solution of 0.5 *N* HF). A mechanical glass stirrer kept the solution in motion. Some runs were made in a beaker coated on the inside with nail-polish, to test if appreciable amounts of polonium are adsorbed onto the glass walls. Comparison with runs made in uncoated beakers showed this not to be the case. The electrodes were both of platinum. The cathode was a round disc (diameter 2.5 cm), painted on one side with nail-polish to ensure polonium deposition on one side only. Alpha measurements after the electrolysis runs showed that no polonium is adsorbed on the nail-polish coated surface. The anode was a platinum wire parallel with and at about 2 cm from the cathode. The voltage across the electrodes was 5 V, and the current density approximately 50 mA/cm² in all experiments, except where otherwise indicated. This relatively high current-density was used in order to increase the speed of the polonium deposition. Working with longer-lived polonium isotopes, a much lower current density is usually preferable (11).

Polonium electrolysis in the absence of wolfram

The results described in this section were made in the absence of wolfram, in order to be able to extend the duration of the electrolysis beyond the time (approx. 20 min), after which a slight deposit of wolfram or impurities on the cathode would have caused erratic results in the alpha-counting of the polonium.

The influence of nitric acid concentration.—The useful concentration range of nitric acid is limited. In the solutions of 20 ml, when one millimole wolfram and 10.7 millimoles (0.54 *N*) hydrofluoric acid is present, the lower limit is 0.14 *N*, corresponding to the minimum amount of nitric acid necessary to dissolve the wolfram target. The upper limit of about 0.7 *N* is set by the pH influence on the polonium electrolysis. Generally, electroplating of polonium is not done from solutions more acidic than 0.7 *N* in HNO_3 (14, 15). Some of our experiments at stronger acid concentrations indicated that the electrodeposition rate could perhaps be increased somewhat. The results obtained were, however, rather erratic at such high acidities and are not

further considered. A further limit is set on the concentration of HNO_3 by the fact that the hydrated wolfram oxide begins to precipitate when the diluted electroplating solution is more than 1.3 N , if other concentrations are kept constant. The effect of the variation of the nitric acid concentration within this range, on the yield of electroplated polonium is shown in Table 1, and from the curves in Fig. 1. In all tables the results are approximated to the nearest half per cent.

Table 1. Percentage of polonium electrolytically deposited after different time-intervals and at varying HNO_3 concentrations, with a constant HF normality of 0.54.

Duration of run (in min)	HNO_3 concentration (normality)		
	0.14	0.35	0.71 ^a
5	9.5	10	9
10	14	16.5	21.5
15	15	30	33
20		34	41
25		42	
30	19		54
35		55	
45	21		

^a Average of two runs.

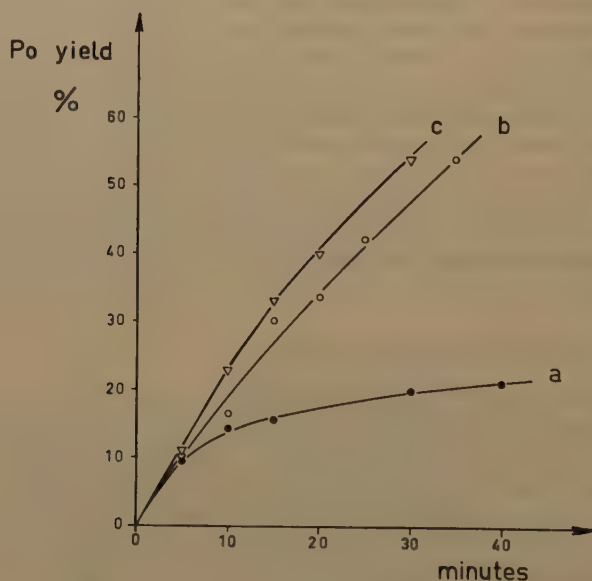


Fig. 1. Percentage of electrodeposited Po as a function of time and varying HNO_3 concentrations. (HF concentration constant at 0.54 N .)

(a) ●, 0.14 N HNO_3 ; (b) ○, 0.35 N HNO_3 ; (c) ▽, 0.71 N HNO_3 .

The influence of hydrofluoric acid concentration.—To investigate quantitatively the influence of the concentration of hydrofluoric acid on the yield of polonium elec-

trolytically deposited, a series of runs was made in which the nitric acid concentration was kept constant at 0.71 *N*. The results given in Table 2 were obtained.

Table 2. Percentage of polonium electrolytically deposited after different time-intervals and at varying HF concentrations, with a constant HNO₃ normality of 0.71.

Duration of run (in min)	HF concentration (normality)				
	0.00	0.27	0.54	1.1	2.2
5	10	14	11.5		
10	24.5	22	23	10	7
15			33		
20	38	39	40		
25				23	12.5
30	50	45			
35			55		
40	60			38	17
55			64.5	53	25

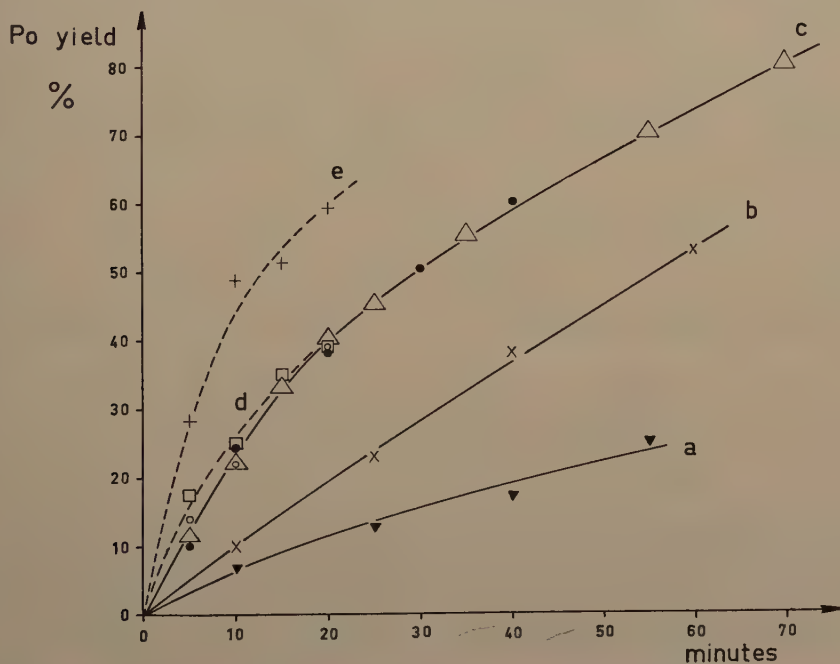


Fig. 2. Percentage of electrodeposited Po as a function of time and varying HF concentrations. (HNO₃ concentration constant at 0.71 *N*.)

In the absence of wolfram: Full-line curves, (a) ▼, 2.2 *N* HF; (b) ×, 1.1 *N* HF; (c) Δ, 0.54 *N* HF; ○, 0.27 *N* HF; ●, HF-free. In the presence of wolfram: Dashed curves, (d) □, 0.54 *N* HF, run at 5 V; (e) +, 0.54 *N* HF, run at 6 V.

From Tables 1 and 2 it is seen that in each series a run with the normalities 0.71 in HNO₃ and 0.54 in HF was made. The results are in good agreement. The curves show that, from about 0.5 *N* HF, the yield-percentage of polonium deposited on

the cathode decreases with increasing HF concentration. This decrease is probably due partly to the higher acidity, and partly to increased complex formation between the polonium and the hydrogen fluoride. In solutions of the HF concentrations below 0.54 *N*, no change in the yields of polonium deposition, within the experimental errors, could be found.

Polonium electrolysis in the presence of wolfram

Three electrolysis runs were made as described above, using solutions containing one millimole of wolfram dissolved in 1.0 ml 10.7 *N* HF and 1.0 ml 14.2 *N* HNO₃. 2 ml of a 1 % tartaric acid was added, and the solution was diluted to 20 ml with water. Finally, a measured amount of polonium tracer was added. Taking into consideration the amount of acids consumed during the dissolution of the wolfram, the solution to be electrolysed was less than 0.54 *N* in HF and 0.71 *N* in HNO₃. The normalities of the free acids were estimated to be near 0.35 *N* in HF and 0.60 *N* in HNO₃. Thus, the acid composition of the wolfram solution is still within the optimum range, according to the previous experiments. For this reason no further experimental work was done to determine accurately the concentration of the free acids. It also appeared unnecessary to vary the acid concentration in the wolfram-containing solutions, since no real improvement could be expected, as is seen from the trends in the wolfram-free solutions. The reproducibility of the deposition as a function of the electrolysis duration is seen in Table 3 from the percentage-yields of three similar runs.

Table 3.

Duration of run (in min)	Run no. . . .	Yield % polonium obtained at 5 V			at 6 V	Yield % bismuth at 5 V
		1	2	3		
5		17	17	18	28	8
10		31	24	26	48.5	11.5
15		41.5	37	29.5	51.5	
20		44.5	39		59.5	

In the three runs made at 5 V, the cathode developed a very slight blue colour after 15 minutes' electrolysis. This occurred also in a trial run made with a solution containing 0.2 % tartaric acid. This minute deposit was probably responsible for the large variations in the amounts of polonium counted after 15 and 20 minutes.

The current-density was also here in all cases close to 50 mA/cm².

It appears that the presence of wolfram in the solutions enhances the deposition yield of polonium during the first 5 minutes by about 6 %. Yields after more than 10 minutes showed no deviations from those obtained in the wolfram-free solutions (see Fig. 2.)

In an attempt to increase the speed of the polonium deposition, a run was made under the same conditions as described above, varying only the voltage across the electrodes to 6 V, and the current-density to about 80 mA/cm². The increased rate of electroplating is seen by comparing the yield percentages in Table 3. This

improvement is, however, counterbalanced by the increased deposited film which impairs the alpha-counting already after 5 minutes' run. Since in this case the dependence of the results on the experimental conditions would be more critical, the reliability of the separation would be decreased. The lower voltage of 5 V is therefore preferred for the polonium deposition in the present case.

Contamination by bismuth had to be avoided since, as was mentioned previously, it may be expected that neutron-deficient bismuth isotopes, having decay properties comparable with those of the polonium isotopes produced, would be formed together with the polonium in the reaction of neon ions on wolfram. To test the percentage yield of bismuth electroplated simultaneously with the polonium, a solution containing a measured amount of purified Bi^{207} was electrolysed under the same conditions as those described for the three polonium runs above. The results are given in Table 3. From these it can be seen that a further quick separation of polonium from the simultaneously electroplated bismuth may be necessary. The volatilization method should be well applicable here because of its rapidity.

From the trend of the influences studied above, on the amount of polonium deposited electrolytically, the recommended solutions are 0.2 to 0.5 *N* in HF, 0.4 to 0.7 *N* in HNO_3 and 0.1 % in tartaric acid, for a concentration of 50 millimoles wolfram per litre.

Determination of optimum electrolysis duration and maximum obtainable yield

Since the final yields of short-lived polonium isotopes were low, it was important to find the optimum duration for the electrolysis. This was done as shown in Fig. 3, where the decay curve (*a*) for an isotope with an assumed half-life of ten minutes is plotted from time $t = 0$, the end of the cyclotron bombardment. The electrolysis yield curve (*c*) from Fig. 2 is also shown in Fig. 3 (*c*).

As the demounting of the target from the cyclotron probe and the dissolution of the wolfram foil takes a certain time, the electroplating is assumed to be started at time t_s .

For a long-lived isotope such as Po^{210} , the electrolysis yield obtained on the platinum plate serving as cathode is thus plotted as the ordinate of the yield curve, which increases from t_s to the end of the electrolysis at e.g. time t_e .

The preparation of the sample for counting, i.e. washing, drying, volatilization if necessary, and mounting in the counting device is finished at time t_m , say.

If volatilization is not carried out, the yield for an isotope, long-lived compared with the separation time, remains constant during the time interval t_e to t_m , which is here taken to be five minutes. In Fig. 3, one thus arrives at the point *D*, which describes the dotted curve (*d*), when the duration of the electrolysis is varied. For short-lived isotopes there is, of course, a pronounced optimum duration for the electrolysis.

The yield curve (*c*) is approximately described by the exponential function $A_{\lambda=0}$

$$A_{\lambda=0} = 1 - e^{k(t-t_s)},$$

where k is a constant. One can deduce that the electrolysis yield for a short-lived isotope in this particular case is obtained by simply multiplying the yield after decay at a given time t with the value of $A_{\lambda=0}$ at that time. The total yield of electrolysis and decay is thus

yield % for decay
and electrolysis

final yield %

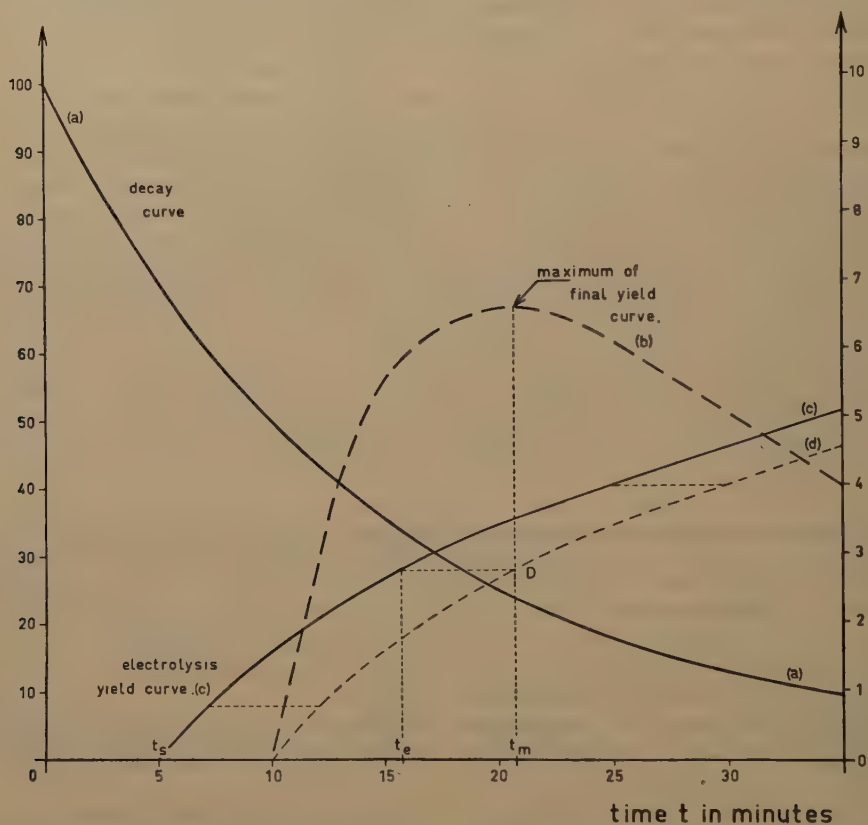


Fig. 3. Graphical representation for the determination of the optimum electrolysis duration in the separation of a polonium isotope having an assumed half-life of 10 minutes. The final yields indicated according to the right-hand ordinate, make no allowance for losses during a re-separation by volatilization. The symbols are explained in the text.

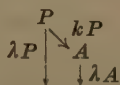
$$A = e^{-\lambda t} (1 - e^{-k(t-t_s)}).$$

where λ is the decay constant for the radioisotope.¹

¹ The pertinent differential equations are

$$\frac{dP}{dt} = -\lambda P - kP \quad \text{and} \quad \frac{dA}{dt} = -\lambda A + kP,$$

where P is the amount of polonium in solution and A that on the cathode, as illustrated by the scheme



The simplicity of the final formula depends on the deposition rate being of the first order.

By derivation, A is found to have its maximum at the time t_e where

$$t_e = t_s - \frac{\ln \frac{\lambda}{\lambda + k}}{k}.$$

The value of λ for the decay curve used as an example in Fig. 3 is 0.0693 min^{-1} , and that for k for the first part of the electrolysis yield curve is approximately 0.038 min^{-1} . This gives $t_e = 16.6$ minutes, giving an optimum duration for electrolysis of about 11.6 minutes.

A convenient way to determine t_e and simultaneously the maximum obtainable yield, $A_{\max}$, at the time t_m , when counting is started, is the following: Multiply the graphically obtained values of the decay curve ordinates and the corresponding yield values for curve (d) at varying times. The dotted final yield curve (b) in Fig. 3 is thus obtained.

The maximum of this curve is $A_{\max}$ (the right-hand ordinate axis is used) and the corresponding time is t_m , from which t_e is obtained by subtracting the separation time. The optimum electrolysis duration is here found to be 10.8 minutes. It should be stressed, however, that this graphical method is strictly valid only in cases for which the electrodeposition function has a rigid exponential form. In all other cases the method can give only approximate values.

Conclusions

The relative applicability of each of the two separation methods described in this paper, or a combination of them, which would lead, in any particular case, to the most convenient preparation of the polonium samples, must be judged from the decay properties of the nuclides formed under the given conditions of irradiation.

Thus, in the present case, for half-lives of the order of two minutes or more, volatilization was applicable. For isotopes having half-lives of ten minutes or more, an initial separation by electrolysis was convenient. This latter method has the advantage that all polonium present in the target is brought into solution, thus giving the possibility of obtaining a higher final yield within the duration of one half-life. Further, the evaporation from the cathode after the electrolysis was found to be neater than directly from the target.

If, in a certain case, a volatilization following the electrolysis is proposed, a higher voltage could be applied for quicker electrodeposition, since the increase in the disturbing contaminations simultaneously deposited with the polonium, would not be evaporated with it. If the polonium isotopes have a sufficiently long half-life (30 minutes or more), a lower current-density is to be recommended, thus generally improving the selectivity of the separation.

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REFERENCES

1. H. ATTERLING, W. FORSLING, and B. ÅSTRÖM, Proceedings of the International Conference on Nuclear Reactions, Amsterdam 1956, p. 1193.
2. D. G. KARRAKER, A. GHIORSO, and D. H. TEMPLETON, Phys. Rev. *83*, 390 (1951); see also W. W. MEINKE, Chemical procedures used in bombardment work at Berkeley, UCRL-432, procedure 84-3 by G. W. BARTON.
3. D. G. KARRAKER and D. H. TEMPLETON, Phys. Rev. *81*, 511 (1951); see also W. W. MEINKE, Chemical procedures used in bombardment work at Berkeley, UCRL-432, procedure 84-1 by D. KARRAKER.
4. K. F. CHACKETT, J. H. FREMLIN, and D. WALKER, Phil. Mag. *7*: *45*, 173 (1954).
5. R. B. LEACHMAN, H. ATTERLING, and B. ÅSTRÖM, Proceedings of the International Conference on Nuclear Reactions, Amsterdam 1956, p. 1191.
6. H. ATTERLING, W. FORSLING, and B. ÅSTRÖM, Arkiv för Fysik, to be published.
7. A. C. WAHL and N. A. BONNER, Radioactivity Applied to Chemistry. New York 1950, table 6 D, p. 440 f.
8. J. HOLLANDER, Thesis, UCRL-1396, 9 (1951).
9. GMELIN, Handbuch der anorganischen Chemie, Syst. Nr. 12 (Berlin), 1941, p. 80.
10. M. HAÏSSINSKY, Radioactivité II, Electrochimie des substances radioactives. Paris 1946, p. 47.
11. G. T. SEABORG, J. J. KATZ, and W. M. MANNING, The Transuranium Elements, vol. *II*, 1949, p. 1174.
12. K. W. BAGNALL and D'EYE, J. Chem. Soc. *1954*, 4295.
13. I. PERLMAN and J. O. RASMUSSEN, UCRL-3424, table I, p. 15, or Vol. 42 of Handbuch der Physik, Berlin, in print.
14. E. BLEULER, and G. J. GOLDSMITH, Experimental Nucleonics. New York, 1952, p. 41.
15. H. RAUDNITZ, Ber. Wien. Akad. *136*, *IIa*, 450 (1927).
16. G. v. HEVESEY and A. GUENTHER, Z. anorg. Ch. *194*, 165 (1930).

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Resolution and configuration of 3-thienylsuccinic acid

By SALO GRONOWITZ

With 2 figures in the text

In connection with work on optically active thiophene compounds (1) 3-thienylsuccinic acid (3-TS) has been resolved into its antipodes. The dextrorotatory enantiomorph was obtained through fractional crystallization of the cinchonidine salt and the levorotatory acid was obtained in the same manner from the $(-)\text{-}\alpha\text{-phenylethylamine}$ salt. As both methods give the same maximum activity it is justifiable to assume that the preparations having a m.p. 170–173 (dec.) represent the optically pure antipodes. Like the 2-isomers (2) the active as well as the inactive acids melt with decomposition. The melted acids crystallize when allowed to cool, but when heated again they show a broad melting point interval about 150–160°. It is also possible that the 3-thienylsuccinic acids, like the 2-isomers (3) occur in several modifications. However, no efforts were made to isolate and characterize them.

In order to determine the absolute configuration of the active 3-thienylsuccinic acids, the Fredga method of quasi-racemates (4) was applied so as to connect this acid sterically with 2-thienylsuccinic acid (2-TS) and phenylsuccinic acid (PS) the absolute configurations of which are known (3).

The 3-thienylsuccinic acids are, as mentioned above, unstable at their melting points, so the traditional thermal analysis for detecting quasi-racemates seemed not feasible. The phase analysis was therefore performed by X-ray powder photographs, which as the present author has pointed out (1) are very useful for the demonstration of quasi-racemates, especially when the structural differences do not exceed the limits of isomorphic exchangeability. Infrared spectroscopy in KBr-phase (5) was used also for the same purpose.

Inactive as well as active 3-thienylsuccinic acid have the same melting points and the powder photographs are very similar (Fig. 1), indicating that the inactive acid is a solid solution (pseudo racemate) of the antipodes. In addition the IR-spectra of the active and inactive acids are very similar which also shows that the inactive acid is a solid solution of the antipodes. True racemates usually have IR-spectra which exhibit considerable differences from the spectra of the antipodes. Inactive 3-thienylsuccinic acid is as could be expected isomorphous with the 2-isomer and has a powder photograph very similar to this acid (Fig. 1). This is also evident from the fact that 3-TS does not depress the melting point of 2-TS. A mixture of these acids has a melting point interval between that of 2- and 3-TS.

Pettersson (3) has found that racemic 2-thienylsuccinic acid is a solid solution of a very unstable third modification of the antipodes. It is highly improbable that a quasi-racemate could be formed between two components which have no tendency

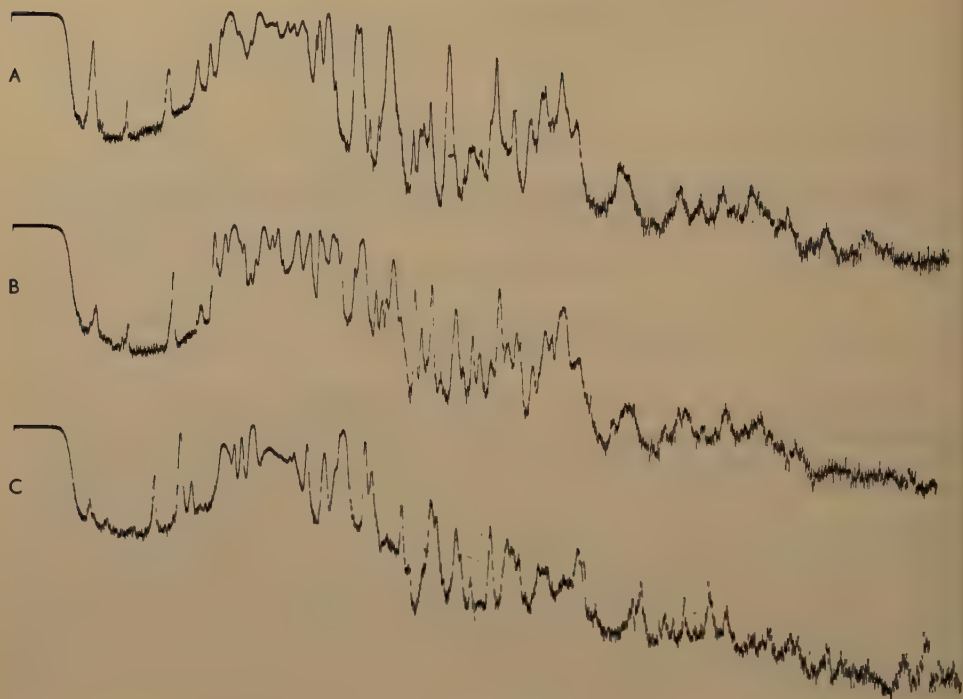


Fig. 1. Photometer curves of X-ray powder photographs of (a) inactive 2-thienylsuccinic acid, (b) inactive 3-thienylsuccinic acid, (c) (+)-3-thienylsuccinic acid.

for racemate formation. It was thus not possible to detect any crucial differences in the X-ray powder photographs or IR-spectra of an equimolar mixture of (+)-2-T and (+)-3-T and of (–)-2-T and (+)-3-T. It is interesting to note that in this case it is not the structural differences between the substances investigated which sets the limits for the quasi-racemate method, but the great similarity which results in a tendency for solid solution formation. This tendency obviously is so great that it even blots out the sterical differences.

However, Pettersson (3) has found through an investigation of the powder photographs that phenylsuccinic acid is a true racemate and that it gives a quasi-racemate with 2-thienylsuccinic acid. (It should be noted that such conclusions must be drawn with care, as the existence of polymorphic forms of the antipodes complicates the situation. The inactive form can for instance be considered as a true racemate in relation to one of the antipodes and a pseudo racemate in relation to the other form.) The system PS-3-TS was therefore investigated. The powder photographs of an equimolar mixture of (+)-3-TS and (–)-PS differs from that of active 3-TS as well as from active PS. The equimolar mixture of the dextrorotatory forms of these acids gives powder photograph which is very similar to active phenylsuccinic acid. This shows that the first-mentioned mixture very probably constitutes a quasi-racemate, while the second is a solid solution of (+)-3-TS in (+)-PS (Fig. 2). In the earlier investigated case of the 2- and 3-thienylsuccinic acid and benzylsuccinic acid (1) the

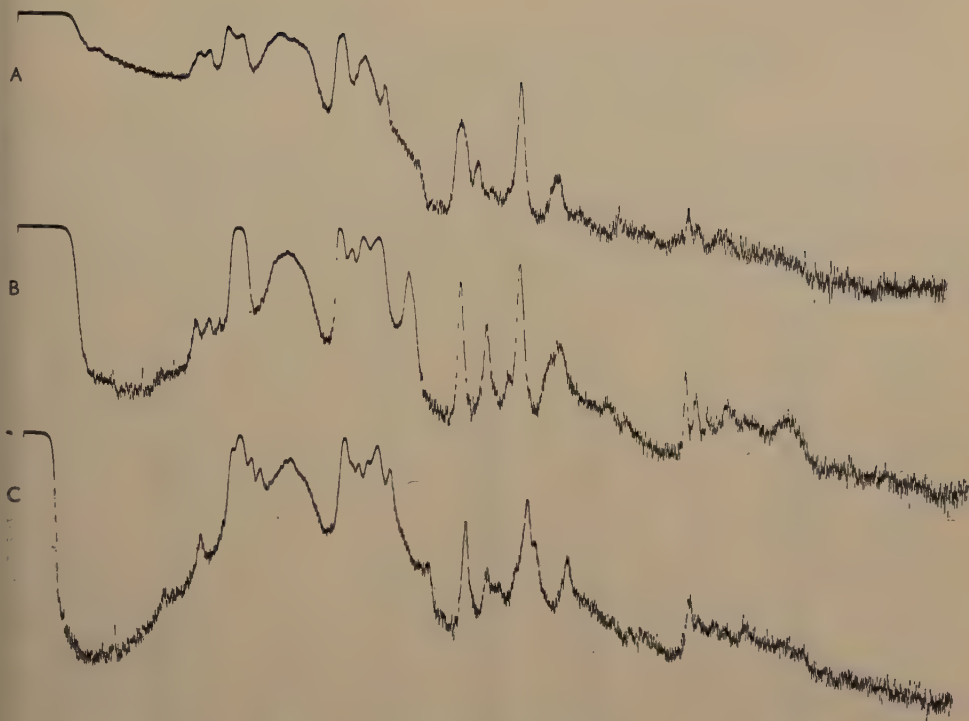


Fig. 2. Photometer curves of X-ray powder photographs of (a) (+)-phenylsuccinic acid, (b) equimolar mixture of (+)-phenylsuccinic acid and (+)-3-thienylsuccinic acid, (c) equimolar mixture of (-)-phenylsuccinic acid and (+)-3-thienylsuccinic acid.

quasi-racemates were isomorphous with the racemic forms of these acids. This is not the case in the system 3-TS-PS, neither could it be expected as inactive PS and TS are not isomorphous.

In order to confirm these results the method of Rosenberg and Schotte (5) which uses the IR spectra of the substances and their equimolar mixtures in the solid state has been tried. The method was first tested with the systems of 2- and 3-thienylsuccinic acid both of which are true racemates and form quasi-racemate. The existence of the racemates and the quasi-racemate between antipodes of opposite direction of rotation has earlier been demonstrated with melting-point diagrams and X-ray powder photographs. It was found that the spectrum of an equimolar mixture of (+)-2-thienylsuccinic acid and (+)-3-thienylsuccinic acids showed the maxima of (+)-2-thienylsuccinic acid as well as (+)-3-thienylsuccinic acid (Table 1). It was interesting to note that the spectra of (-)-2-thienylsuccinic acid and (+)-3-thienylsuccinic acid was additively composed of the spectra of rac.-2- and -3-thienylsuccinic acid (Table 2). This was not the case in the systems investigated by Schotte and Rosenberg. This result confirms the usefulness of this method to detect quasi-racemates although Pettersson (6) is of the opinion that this method is of lesser generality than the thermal and X-ray methods.

Table 1. The infrared absorption bands of (+)-2-thenylsuccinic acid, (+)-3-thenylsuccinic acid and an equimolar mixture of these acids.

b = broad, vs = very strong, s = strong, m = medium, w = weak, sh = shoulder.

(+)-2-Thenylsuccinic acid			(+)-3-Thenylsuccinic acid			(+)-2-Thenylsuccinic acid and (+)-3-thenylsuccinic acid		
Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band
3.4	2941	sb	3.4	2941	sb	3.4	2941	sb
3.8	2631	b	3.8	2631	b	3.8	2631	b
5.85	1709	vs	5.85	1709	vs	5.85	1709	vs
6.98	1433	s	7.00	1429	s	7.00	1429	s
7.13	1403	m	7.11	1406	m	7.12	1404	m
7.36	1359	w	7.36	1359	w	7.36	1359	w
7.77	1287	s	7.50	1333	w	7.49	1335	w
8.01	1248	w	7.74	1292	s	7.74	1292	s
8.15	1227	m	8.05	1242	w	8.05	1242	b sh
8.37	1195	m	8.10	1235	sh	8.15	1227	m
8.51	1175	m	8.17	1224	w	8.37	1195	m
9.27	1079	w	8.38	1193	m	8.51	1175	m
9.35	1070	w	8.74	1144	m	8.73	1145	w
9.65	1036	w	9.34	1071	w	9.3	1075	wb
10.18	982	w	10.15	985	w	9.66	1035	w
10.7	936	b	10.7	936	b	10.15	985	w
11.51	869	m	11.46	873	m	10.7	936	b
11.75	851	w	11.74	852	m	11.45	873	sh
11.88	842	m	11.90	840	s	11.50	870	m
12.10	826	w	12.83	779	vs	11.75	851	m
14.30	699	vs	14.6	685	b	11.90	840	m
						12.10	826	w
						12.83	779	s
						14.30	699	s
						14.60	685	b

The spectra of an equimolar mixture of (+)-3-TS and (-)-PS is different from the spectra of the components. In the region between 7-9 μ which according to Schotte and Rosenberg (5) is the most useful, there exist several differences. A new band appears at 8.04 μ which is not present in the components and the strong band at 8.56 μ in (+)-PS and 8.59 μ in (-)-TS has completely disappeared. Differences exist, however, also at longer wavelength, as is apparent from Table 3. The spectra of the equimolar mixture of (+)-3-TS and (+)-PS is, however, not completely additively composed of its components. Thus for instance the weak band between 8.10-8.20 μ has become a stronger and sharper band at 8.17 μ . In other parts of the spectrum a broadening of the bands has occurred and the general appearance of the spectrum is that of an impure substance. These deviations from additivity are, however, so insignificant compared with the great deviations in the spectrum of the mixture of (+)-3-TS and (-)-PS, that it seems very probable that the latter mixture constitutes a quasi-racemate. The IR-investigation is thus in agreement with the results obtained from X-ray powder photographs.

Table 2. The infrared absorption bands of rac.-2-thenylsuccinic acid, 3-thenylsuccinic acid and an equimolar mixture of (–)-2-thenylsuccinic acid and (+)-3-thenylsuccinic acid.

For abbreviations see Table 1.

rac.-2-Thenylsuccinic acid			rac.-3-Thenylsuccinic acid			(+) -3-Thenylsuccinic acid and (–) -2-thenylsuccinic acid		
Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band
3.4	2941	sb	3.4	2941	sb	3.4	2941	sb
3.8	2631	b	3.8	2631	b	3.8	2631	b
5.87	1704	sh	5.92	1689	vs	5.83	1715	vs
5.92	1689	vs	6.92	1445	sh	5.90	1695	vs
6.92	1445	sh	7.05	1418	sh	6.92	1445	sh
7.02	1425	sh	7.10	1408	s	7.02	1425	sh
7.11	1406	s	7.52	1330	m	7.10	1408	s
7.52	1330	m	7.60	1316	m	7.52	1330	m
7.63	1311	m	7.75	1290	m	7.62	1312	m
7.78	1285	w	7.81	1280	sh	7.72	1295	sh
8.06	1241	s	7.98	1253	m	7.80	1282	sh
8.20	1220	s	8.10	1235	s	7.98	1253	sh
8.32	1202	w	8.21	1218	vs	8.06	1241	s
8.47	1181	m	8.63	1159	w	8.22	1217	vs
8.83	1133	w	8.72	1147	w	8.31	1203	sh
9.15	1093	w	9.15	1093	w	8.46	1182	m
9.27	1079	w	9.30	1075	w	8.61	1161	sh
9.64	1037	w	10.02	998	w	8.70	1149	sh
9.87	1013	w	10.67	937	m	9.15	1093	m
10.80	926	mb	11.00	909	sb	9.28	1078	w
11.15	896	m	11.50	870	m	9.64	1037	wb
11.55	865	m	11.68	857	m	9.87	1013	sh
11.75	851	w	11.87	842	m	10.10	990	wb
11.88	842	m	12.05	830	sh	10.90	917	sb
12.04	831	m	12.83	779	vs	11.10	901	sb
12.78	782	w	14.22	703	w	11.55	866	mb
13.03	767	w	14.93	670	m	11.88	842	m
14.13	708	vs				12.05	830	sh
14.55	687	w				12.80	781	s
						14.10	709	vs
						14.55	687	wb
						14.93	670	m

The rotatory power of (+)-3-TS was measured in five different solvents. The results thus obtained were practically the same as those with (+)-2-TS in the same solvents (3). These findings make it important to ascertain that the two preparations really are the two compounds 3-TS and 2-TS. The IR-spectra, the melting points, and the methods of preparation (2, 14), however, conclusively show that there is no doubt about the authenticity of the preparations.

The optical rotatory power for 3- and 2-TS was also measured at different wavelengths in the visible spectrum, and the results (Table 5) may indicate that the rotation of 2-TS increases more rapidly than that of 3-TS toward the UV-region. It should be

Table 3. The infrared absorption bands of (+)-phenylsuccinic acid, (+)-3-thienylsuccinic acid, equimolar mixture of (+)-phenylsuccinic acid and (+)-3-thienylsuccinic acid, equimolar mixture of (-)-phenylsuccinic acid and (+)-3-thienylsuccinic acid.

For abbreviations see Table 1.

(+)-Phenylsuccinic acid			(+)-3-Thienylsuccinic acid			(+)·PS and (+)-3-TS			(-)·PS and (+)-3-TS		
Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band	Wave-length (μ)	Wave-numbers (cm^{-1})	Character of the band
3.4	2941	sb	3.4	2941	sb	3.4	2941	sb	3.4	2941	mb
3.8	2631	b	3.8	2631	b	3.8	2631	b	3.8	2631	b
5.90	1695	vs	5.85	1709	vs	5.80	1724	vs	5.87	1704	vs
6.26	1597	m	5.92	1689	vs	5.85	1709	vs	6.25	1600	w
6.33	1580	w	6.55	1527	w	5.91	1692	vs	6.30	1587	w
6.70	1493	m	7.11	1406	s	6.26	1597	w	6.88	1453	m
6.88	1453	m	7.80	1282	s	6.33	1580	w	7.05	1418	s
7.07	1414	s	8.10	1235	w	6.70	1493	m	7.40	1351	w
7.70	1299	s	8.42	1188	s	6.88	1453	sh	7.62	1312	s
7.90	1266	w	8.59	1164	s	7.12	1404	s	7.75	1290	sb
8.12	1232	w	9.26	1080	m	7.75	1290	sb	7.85	1274	sb
8.37	1195	m	10.7	935	b	8.17	1224	m	8.04	1244	s
8.56	1168	s	11.02	907	m	8.37	1195	mb	8.17	1224	m
9.27	1079	m	11.57	864	m	8.58	1166	s	8.28	1208	w
9.33	1072	w	12.03	831	w	9.27	1079	m	8.42	1188	s
9.74	1027	w	12.50	800	w	9.33	1072	w	9.25	1081	m
9.99	1001	w	13.20	758	s	9.74	1027	w	9.73	1028	w
10.70	935	b	14.95	669	s	10.04	996	w	10.05	995	w
12.22	818	w				10.15	985	w	10.75	930	b
13.21	757	m				10.70	935	b	11.02	907	sh
13.80	725	s				11.03	907	m	11.57	864	w
14.32	698	s				11.57	864	m	11.77	850	w
14.48	691	s				11.85	844	w	11.87	842	w
						12.52	799	w	13.02	768	s
						13.00	769	mb	13.87	721	s
						13.20	758	m	14.45	692	s
						13.80	725	mb			
						14.35	697	mb			
						14.97	668	m			

Table 4. Optical activity of 2-TS and 3-TS.

Solvent	[M] _D ²⁵ of acid	
	(+)-2-TS	(+)-3-TS
Water (neutr.)	81.8°	80.6°
Ethanol	205.6°	204.6°
Acetic acid	225.6°	225.2°
Acetone	249.2°	247.4°
Ethylacetate	252.4°	256.4°

noted that this very similar rotatory power in the visible region for 2- and 3-TS acid is not general for all isomeric optically active thiophene compounds. The optical rotatory power for 2- and 3-thenylsuccinic acid shows a remarkable parallelism in different solvents but the molar rotations are not of the same magnitude (1). In this case, however, the thienyl radical is not directly attached to the asymmetric carbon atom and it will be interesting to find out if other isomeric optically active thiophene compounds display the same phenomena when the thienyl radical is directly attached to the asymmetric center.

From the results obtained it can be concluded that 3-thienylsuccinic acid and phenylsuccinic acid with the same mode of rotation also have the same configuration. Pettersson (3) has connected phenylsuccinic acid via quasi-racemate to 2-thienylsuccinic acid, and the latter to the glyceraldehyde system through desulphurisation with Raney-Nickel according to the general method given by Fredga (7). He found that (+)-phenylsuccinic acid is represented by stereoformula I. Consequently (+)-3-thienylsuccinic acid has the corresponding formula II.



In an earlier investigation (8) the present author has studied the difference in the conjugative electron-donating properties (+M-effect, according to Ingold's terminology) of the thiophene nucleus, when attached through the 2- and 3-position. A study of the racemization of optically active thiophene compounds having hydrogen at the asymmetric center gives an opportunity to study the electron-attracting properties (-I, -M effect) of the 2- and 3-thienyl group. Ever since the investigations by Ramberg and his school (9) and by Ingold and his coworkers (10) on the bromination, deuteration and base-catalysed racemization of optically active compounds having hydrogen at the asymmetric center, it is generally believed that the rate-determining step of the racemization of such compounds is due to ionization of the hydrogen. The anion formed racemizes readily since it can pass into a symmetrical form. [For extensive discussions on the mechanism of prototropy cf. Dewar (11) and Ingold (12).] The relation between structure and rate of racemization and other

prototropic reactions is well known and it has been found that the rate increases with the electron-attracting properties of the other substituents at the asymmetric center. An electron-attracting aromatic substituent may of course be active both through its inductive and conjugative effect. It seems, however, highly probable that it is the large inductive effect of aromatic substituents, which is most important, as the conjugative effect can first come into play with the weakening of the C-H bond in the transition state which makes electrons available for conjugation. The conjugation is further hindered through cross-conjugation with the other activating group such as carbonyl.

Pettersson (2) has found that 2-TS is almost completely racemized after refluxing with 2 *N* sodium hydroxide. Under similar conditions PS is not racemized at all (13). In a preliminary racemization experiment the present author has now found that under the same conditions 3-TS is racemized about 20 %. These results show that the electron-attracting properties increase in the order phenyl < 3-thienyl < 2-thienyl.

Experimental

Preliminary test on resolution—0.0025 moles of optically inactive acid and 0.005 moles of the base were dissolved in 25 ml of dilute ethanol. The solutions were allowed to stand in the refrigerator and some water gradually added until crystals were formed. Experiments with (+)-phenylethylamine were made in ethyl acetate. The results are given below.

Base	$[\alpha]_D^{25}$ of acid in acetone
Brucine	+ 10.5°
Strychnine	+ 17.4°
Cinchonidine	+ 82.7°
Quinine	+ 6.5°
(+)- α -phenylethylamine	+ 14.0°

Optical resolution of 3-thienylsuccinic acid.—20 g (0.1 moles) of 3-thienylsuccinic acid (8) and 60 g (0.2 moles) cinchonidine were dissolved in 800 ml 50 % ethanol and the solution left in a refrigerator for 24 hours. The precipitate was filtered off, washed with cold dilute ethanol and dried. About 0.3 g of the salt was set apart, the acid isolated and the optical activity measured in ethanol. The remaining salt was recrystallized from dilute ethanol and the process repeated until the optical activity of the acid remained constant. The course of the resolution is seen below.

Crystallization	1	2	3	4	5
g salt	42	26	19	14.5	8
ml 50 % ethanol	800	700	400	350	300
$[\alpha]_D^{25}$ of acid	+ 65°	+ 92°	+ 102°	+ 103°	+ 103°

The cinchonidine salt obtained in the course of resolution as needle bundles, was not further examined.

The mother liquor from the first crystallization with cinchonidine was evaporated at room temperature and 38 g salt was obtained. This salt yielded 9 g acid, having $[\alpha]_D^{25} = -62^\circ$ in ethanol. This acid was dissolved in a mixture of 540 ml ethyl acetate and 90 ml methanol and 10.9 g of (–)- α -phenylethylamine was added. The progress of the resolution is seen below.

Crystallization	1	2	3	4	5	6
ml, ethylacetate	540	550	440	220	150	105
ml, methanol	90	340	270	135	90	65
g salt	17	11.5	7.2	4.9	3.4	2.4
$[\alpha]_D^{25}$ of acid	-78°	-92°	-99°	-101°	-102°	-102°

The phenylethylamine salt obtained in the course of the resolution was not further examined.

(+)-3-*Thienylsuccinic acid*.—The cinchonidine salt (7.7 g) was decomposed with 2 *N* sulphuric acid and the active acid extracted with ether. The ether was evaporated at room temperature and the acid was recrystallized two times from water. The yield of pure acid was 1.5 g and the m.p. 170–173°. The acid crystallized in colourless plates.

0.06562 g : 8.90 ml 0.0734 *N* NaOH. $C_8H_8O_4S$ (200.0). Equiv. wt. calc. 100.0, found 100.5. Weighed amounts of acid were made up to 10.00 ml with different solvents and the optical activity measured. In the case of water the acid was neutralized with 0.1 *N* NaOH and made up to 10.00 ml.

The results are given below.

Solvent	mg of acid	$2[\alpha]_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Ethanol	97.00	+ 1.985°	+ 102.3°	+ 204.6°
Ethyl acetate	92.45	+ 2.33°	+ 128.8°	+ 257.6°
Acetone	79.88	+ 1.98°	+ 123.7°	+ 247.4°
Acetic acid	87.25	+ 1.965°	+ 112.6°	+ 225.2°
Water (ion)	67.60	+ 0.545°	+ 40.3°	+ 80.6°

(-)-3-*Thienylsuccinic acid*.—2.1 g of the (-)-phenylethylamine salt was decomposed with 2 *N* sulphuric acid and the active acid extracted with ether. The ether was evaporated and the acid recrystallized in the same way as the antipode which it resembles in appearance and properties. The yield was 0.85 g having m.p. 170–173°.

65.29 mg: 8.84 ml 0.0734 *N* NaOH, $C_8H_8O_4S$ (200.0). Equiv. wt. calc. 100.0, found 100.6. 0.10363 g made up to 10.00 ml with ethanol. $2[\alpha]_D^{25} = -2.125^\circ$, $[\alpha]_D^{25} = -102.5^\circ$.

The optical rotations of 0.1498 g (+)-2-TS in 20 ml ethanol and 0.1658 g (+)-3-TS in 20 ml ethanol were measured at different wavelengths. The results are given in the Table 5.

Table 5. Rotation dispersion of 2-TS and 3-TS.

Wavelength Å	2-TS		3-TS	
	$2[\alpha]^{25}$	$[\alpha]^{25}$	$2[\alpha]^{25}$	$[\alpha]^{25}$
6438	+ 1.652°	+ 99.6°	+ 1.509	+ 100.7
5893	+ 2.043°	+ 123.2°	+ 1.828°	+ 122.0°
5461	+ 2.484°	+ 149.8°	+ 2.211°	+ 147.6°
5086	+ 2.958°	+ 178.4°	+ 2.621°	+ 174.9°

Optical activity measurements.—A polarimeter Barfit M 413 from Hilger and Watts was used with sodium, cadmium and quicksilver lamps and appropriate filters.

Melting-points.—The melting-points were determined in a Kofler hot-stage microscope. The substance was placed in the microscope about ten degrees under their melting-points and heated at the rate of 2°/min.

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X-ray powder photographs.—X-ray powder photographs were taken in a focusing camera of the Gunier (9) type with Cu K_{α} radiation (45 kV, 25 mA). Time of exposure three hours. The preparations were obtained by dissolving weighed amounts of the components in acetone. The solvent was evaporated at room temperature and the residue carefully powdered and dried in a desiccator.

Infrared spectroscopy.—A Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prism was used for the infrared absorption measurements. The preparations were obtained in the same manner as described for the X-ray powder photographs and were examined as solid pressed potassium bromide plates according to the method described by Schiedt and Reinwein (10).

Racemization experiment.—0.57156 g of incompletely resolved (–)-3-TS having $[\alpha]_D^{25} = -91.4^{\circ}$ in ethanol, was made up to 20.00 ml with 2 *N* sodium hydroxide, $2[\alpha]_D^{25} = -2.08^{\circ}$, $[\alpha]_D^{25} = -36.5^{\circ}$. The solution was then heated under a reflux-condensor in a boiling water-bath for 135 minutes. Rapidly cooled to 25° and the optical activity measured $2[\alpha]_D^{25} = -1.63^{\circ}$, $[\alpha]_D^{25} = -28.5^{\circ}$.

SUMMARY

3-Thienylsuccinic acid has been resolved into its antipodes. Its steric relationship to phenylsuccinic acid has been established by the quasi-racemate method and thus it has been connected to the glyceraldehyde system. Its optical rotatory power in five different solvents has been found to be of the same magnitude as the rotatory power of 2-thienylsuccinic acid.

ACKNOWLEDGEMENTS

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REFERENCES

1. GRONOWITZ, S., and LARSSON, S., *Arkiv Kemi* 8, 567 (1955).
2. PETTERSSON, K., *Arkiv Kemi* 7, 39 (1954).
3. PETTERSSON, K., *Arkiv Kemi* 7, 347 (1954).
4. FREDGA, A., *The Svedberg 1884 30/8 1944*. Uppsala 1944, p. 261.
5. ROSENBERG, A., and SCHOTTE, L., *Arkiv Kemi*, 8, 143 (1955).
6. PETTERSSON, K., *Arkiv Kemi* 10, 297 (1956).
7. FREDGA, A., *Arkiv Kemi* 6, 277 (1953).
8. GRONOWITZ, S., and ROSENBERG, A., *Arkiv Kemi* 8, 23 (1955).
9. RAMBERG, L., and MELLANDER, A., *Arkiv Kemi, Mineral. Geol. 11B* n:o 31 (1934).
- RAMBERG, L., and HEDLUND, I., *Arkiv Kemi, Mineral. Geol. 11B* n:o 41 (1934).

10. HSÜ, S., and INGOLD, C. K., *J. Chem. Soc.* 1936, 623.
 HSÜ, S., INGOLD, C. K., and WILSON, C. L., *J. Chem. Soc.* 1938, 78.
11. DEWAR, M. J. S., *Electronic Theory of Organic Chemistry*. Oxford 1952, pp. 97-100.
12. INGOLD, C. K., *Structure and Mechanism in Organic Chemistry*. London 1953, pp. 530-575.
13. WREN, H., and WILLIAMS, H., *J. Chem. Soc.* 109, 572 (1916).
14. GRONOWITZ, S., *Arkiv Kemi* 8, 441 (1955).
15. GUINIER, A., *Ann. Phys.* [11], 12, 161 (1939).
16. SCHIEDT, U., and REINWEIN, H., *Z. Naturforsch.* 7b, 270 (1952).

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Thiophene Chemistry. Part VI

Bromination of thiophene and bromothiophenes

By SVEN-OLOV LAWESSON

With 8 figures in the text

In organic chemistry it has often been fruitful to make analogies. Thus a comparison of the benzene- and the thiophene-halogens has earlier indicated that the constitution of the bromothiophene isomers could be determined. However, there is some uncertainty and as very little is known about the chemistry of the bromothiophenes and as the existing synthetic methods are quite inadequate it seemed worthwhile to make an investigation of the bromination products and to work out newer and better methods to prepare such compounds.

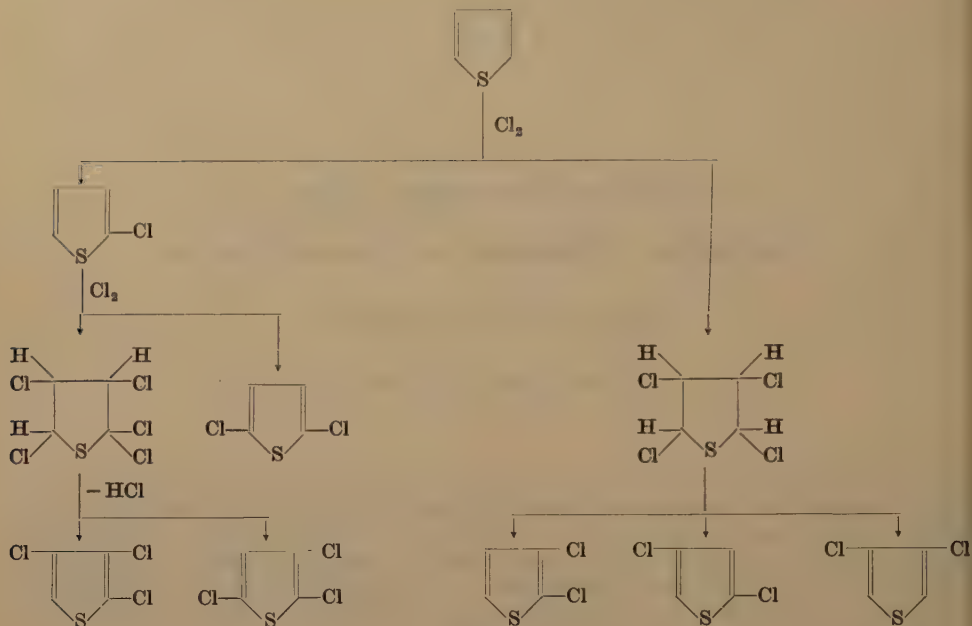
No systematic investigation of the bromination of thiophenes has hitherto been made. However, a great many bromothiophene derivatives have been prepared, since Meyer (1) made the first bromination of thiophene without solvents and thereby obtained 2-bromothiophene and 2,5-dibromothiophene, the product first mentioned only in a very small yield. Thus, 2-bromothiophene was prepared in better yields by bromination in glacial acetic acid (2, 3) and, in benzene (4) but the best result was gained by bromination with N-bromosuccinimide (5) according to the method of Wohl-Ziegler (6). Another method of preparing 2-bromothiophene is to use N-bromoacetamide (7) or cyanogen bromide (8) as bromination agent. Recently a study of the vapour halogenation of thiophene (9) has been published. Here the thiophene was brominated in the gas phase at a temperature up to 750°C. It was found that mono substitution varies from 2-bromo to 3-bromothiophene as the temperature rises. At 750°C 3-bromothiophene was produced in a yield of 16 %. Furthermore it was shown, that the 3-isomer was not obtained by the rearrangement of the 2-derivative and that the new method was of no practical value.

Besides by the above mentioned method of Meyer 2,5-dibromothiophene has been prepared from thiophene, using N-bromoacetamide (7) as bromination agent.

Tribromothiophene, the 2,3,5-isomer, has been obtained by treating 2,5-dibromothiophene (10) with bromine.

Tetrabromothiophene was obtained by further bromination of polybromothiophenes (according to Meyer (11)) and by bromination of thiophene (12).

Hartough and his collaborators (13) have carried out an extensive investigation of the chlorination of thiophene. Eight substitution products were isolated and a ninth, 3-chlorothiophene, was found as traces. At the same time stable chlorine addition products (14) were prepared and these have been assumed to be intermediates in the chlorination. Thus Hartough concludes that the following scheme is valid:



Scheme 1.

This leads to the fairly obvious conclusion that bromine addition compounds of thiophene must exist, but none has hitherto been reported in the chemical literature. Only the substitution products 2-bromo, 2,5-dibromo-, 2,3,5-tribromo- and 2,3,4,5-tetrabromothiophenes have been obtained in the bromination of thiophene. Earlier authors (15, 16) have, however, presumed the formation of addition compounds, on the ground that they must be destroyed by a prolonged heating with alcoholic KOH or NaOH, the brominated product being thus stabilized in this way. Considering that the bond energy of the bromine-carbon bond is lower than that of the corresponding chlorine-carbon bond it can be assumed that a possible bromine-addition product will be less stable and more susceptible to heat than the known chlorine-addition compounds and that the melting point ought to be higher.

A series of bromination experiments has been performed under the most different conditions. The results are given in Table 1. Thus bromination of thiophene without any catalyst is a fast reaction. After the two α -hydrogens have been substituted with bromine, addition ought to dominate substitutions, as on chlorination, and consequently (compare Scheme 1) the dibromothiophene fraction should be a mixture of the four different isomers, and the tribromo fraction should contain 2,3,4-tribromothiophene principally. We have not been able to isolate any stable addition compound however, even if the bromination was performed under the mildest conditions. This result leads to suspect that such addition compounds can only exist as very unstable intermediates. Furthermore the introduction of bromine into the β -positions after the α -positions have been substituted with bromine was found to be very sluggish, but by increasing the temperature or extending the reaction time the product desired is obtained in good yield.

Electrophilic aromatic substitution of thiophene can be pictured in the following way, where $R^{\delta+}$ may be NO_2^+ or Br^+ .

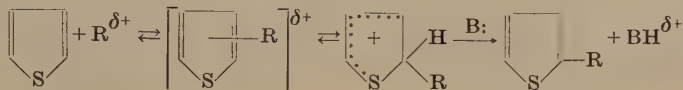


Fig. 1 illustrates the energy relationships. (A is bond formation and B bond scission.)

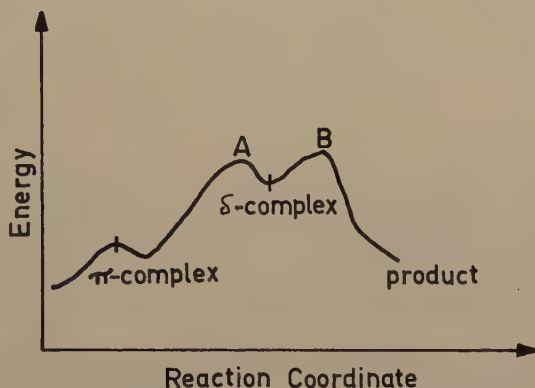


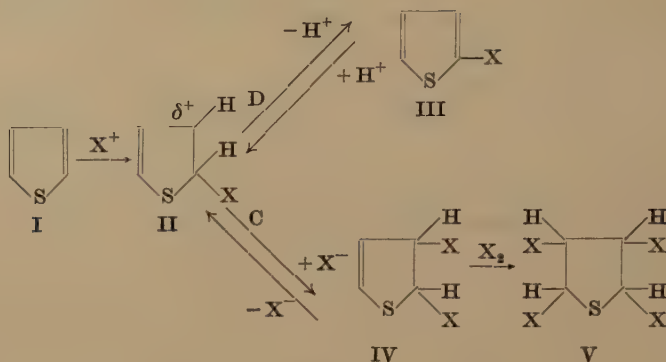
Fig. 1. Relationships during aromatic substitutions.

Dewar (17, 18) is of the opinion that the formation or decomposition of the π -complex is rate-determining while Brown *et al.* (19) claim that the stability of the δ -complex determines the rate. Hitherto, no agreement has been reached as to which step is rate-controlling. However, the rate of formation of the δ -complex is proportional to the concentration of the π -complex. Furthermore, the over-all reaction rate is influenced by the relative heights at A and B. For example, in aromatic bromination and nitration there is no detectable hydrogen isotope effect, while in sulfonation there is a pronounced isotope effect. The problem of the importance of the various steps has been discussed (20, 21).

In the bromination of thiophene or bromothiophene there is a violent evolution of hydrogen bromide and this might indicate that the mechanism for the formation of the intermediate, the so called addition compound. (Stage C in Scheme 2), now accepted for $X = \text{Cl}$, is not valid for $X = \text{Br}$, but that instead there is perhaps principally a mere substitution reaction (Stage D).

The equilibrium conditions do not necessarily render stage C improbable and the addition compound IV may evidently give the normal substitution product without being a real intermediate compound. No addition compound of the IV-type ($X = \text{Cl}, \text{Br}$) has hitherto been isolated. It will, however, add one mole of halogen and form V (only for $X = \text{Cl}$), which has been prepared and investigated by Hartough and coworkers (14).

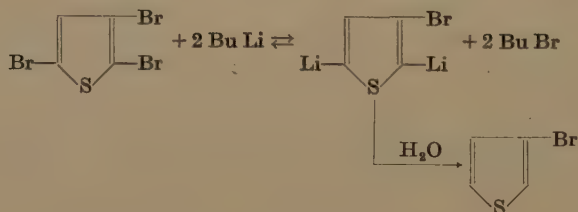
As a logical consequence of our unsuccessful attempts to isolate bromothiophene-addition compounds it may be expected that the bromination of thiophene will give successively 2-bromo, 2,5-dibromo, 2,3,5-tribromo and 2,3,4,5-tetrabromothio-



Scheme 2.

phenes. From Table I it is seen that this has been confirmed. In earlier investigations in thiophene chemistry the differences in reactivity between the α - and β -positions have not always been taken into consideration. Therefore, in order to unequivocally identify the different products formed in the bromination experiments the different isomers have been synthesized by a somewhat more complicated and sometimes indirect method.

3-Bromothiophene (22) was prepared by debromination of 2,3,5-tribromothiophene with butyllithium. According to the yield, the preparation of 3-bromothiophene has been improved by performing the experiment on a larger scale, whereby the loss during the distillation will be relatively smaller. The reaction may be written as follows, and it is of importance to displace the equilibrium to the right. By using



more than 2 mol. of butyllithium per mol. tribromothiophene a yield of 84% was obtained. An excess of lithium reagent is favourable and constitutes no disadvantage because it is impossible to introduce three lithium atoms into the bromoderivative with butyllithium. The reason is that by the strong electrostatic effects of the



lithium atoms, the C_3 carbon will have a negative net charge and a nucleophilic attack there cannot occur. Another advantage with this method over that of Steinkopf is, that the butyl bromide, originally used for the preparation of the butyllithium, is recovered.

Isomer-free 2,5-dibromothiophene was prepared by bromination of 2-bromothiophene with N-bromosuccinimide. Because the sulfur atom activates the α -positions for electrophilic substitutions (23, 24) there ought to be only 2,5-dibromothiophene under these especially mild conditions.

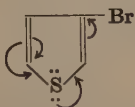
3,4-Dibromothiophene was prepared from 2,3,4,5-tetrabromothiophene by debromination with butyllithium (25).

2,4-Dibromothiophene was prepared from 2,3,5-tribromothiophene by debromination with butyllithium at a low temperature (22, 26).

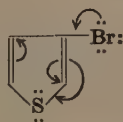
2,3-Dibromothiophene was first prepared by bromination of 2-thiophenecarboxylic acid to 4,5-dibromo-2-thiophenecarboxylic acid and then decarboxylation via the corresponding HgCl-compound (27). In fact this isomer is a compound which requires lengthy preparative procedures. The steps are simple and the yields are all good but a method requiring fewer steps, even if more complex, would be advantageous in the use of this compound in synthesis.

Now 3-bromothiophene is easily available (23) and by brominating it with 2 moles of bromine one obtains only 2,3,5-tribromothiophene, free from the other isomer, because it is difficult to believe, that any bromination would occur at the 4-position. The difference in reactivity between the α - and β -positions is great and Halvarson and Melander (28) have for instance shown in isotopic exchange reactions in thiophene, that the exchange rates of tritium in the 2-position is about 1000 times higher than that in the 3-position.

Bromination of 3-bromothiophene with one mole of bromine may be more complicated. In this case 2,3-dibromothiophene, 2,4-dibromothiophene or a mixture of these two isomers can be obtained. According to the symbolism of Ingold (29), 3-bromothiophene is characterized as $-I + T$. The bromine atom, being strongly negative, pulls electrons away from the carbon atom to which it is attached. The orientation should be meta, with deactivation for electrophilic reagents, and ortho, para with activation for nucleophilic reagents if this displacement of charge was the only factor involved. This may be represented as follows:

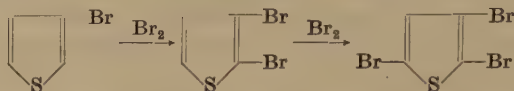


Because the bromine atom has an unshared pair of electrons the tautomeric effect can play an important role and if this was the only factor the orientation would be ortho, para with activation for an electrophilic reagent, and meta with deactivation for a nucleophilic reagent according to:

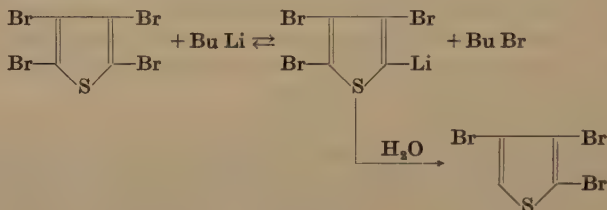


These two conflicting effects make it difficult to predict what would be the orientation for an electrophilic reagent. Experience from other aromatic compounds has shown that the resulting orientation is ortho, para with deactivation and in reference to 3-bromothiophene one can expect that the first bromine atom will enter into

the 2-position. This has been confirmed experimentally and bromination of 3-bromothiophene will thus follow the following scheme:



2,3,4-tribromothiophene was prepared by debromination of tetrabromothiophene with butyllithium according to:



As to the bromination experiments we have found that it is not necessary to stabilize the brominated products by heating with sodium- or potassium-hydroxide in ethanol. It is quite adequate to heat the solution up to 100°C for a couple of hours. If solid NaOH or KOH is used the heating should not extend for days as was earlier claimed but two or three hours is enough.

It has been proposed (30) that the bromination of thiophene occurs analogous to chlorination. In the present investigation this assumption has been shown to be invalid.

Experimental

The infrared spectra were recorded with a Perkin-Elmer spectrophotometer (model 21) equipped with NaCl optics.

Preparation of 3-bromothiophene

To a solution of 100 g (0.31 mol.) of 2,3,5-tribromothiophene in 100 ml of dry ether cooled to 0°C in an ice-water mixture and in a nitrogen atmosphere, 0.7 mol. of butyllithium in 400 ml absolute ether, cooled to a temperature below 0°C, was added in a slow stream under vigorous stirring. The addition was complete after 60 minutes. The resulting yellow emulsion was stirred for another half an hour and then poured into water. The organic layer was separated and the water phase shaken several times with ether. The combined extracts were shaken with water once, dried over sodium sulphate freed from solvent and then the residue distilled. The main fraction, 3-bromothiophene (b.p. 44–46/11 mm Hg, weight 43 g, yield 84 %) was followed by a mixture of 2,3- and 2,4-dibromothiophenes (2 g).

Preparation of 2,5-dibromothiophene

2-Bromothiophene (Eastman) (32.6 g) was refluxed for 30 hours in carbon tetrachloride (150 ml) with N-bromosuccinimide (44 g), purified according to Chapman and Williams (31). The mixture was cooled, filtered, freed from solvent and diluted

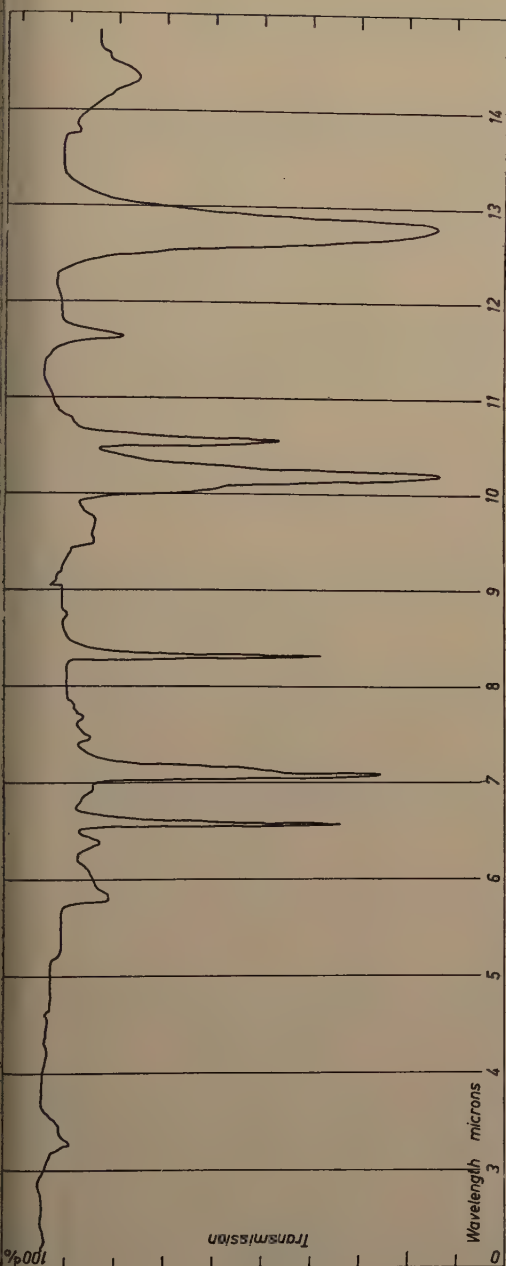


Fig. 2. The infrared spectrum of 2,5-dibromothiophene, prepared from N-bromosuccinimide (liq.).

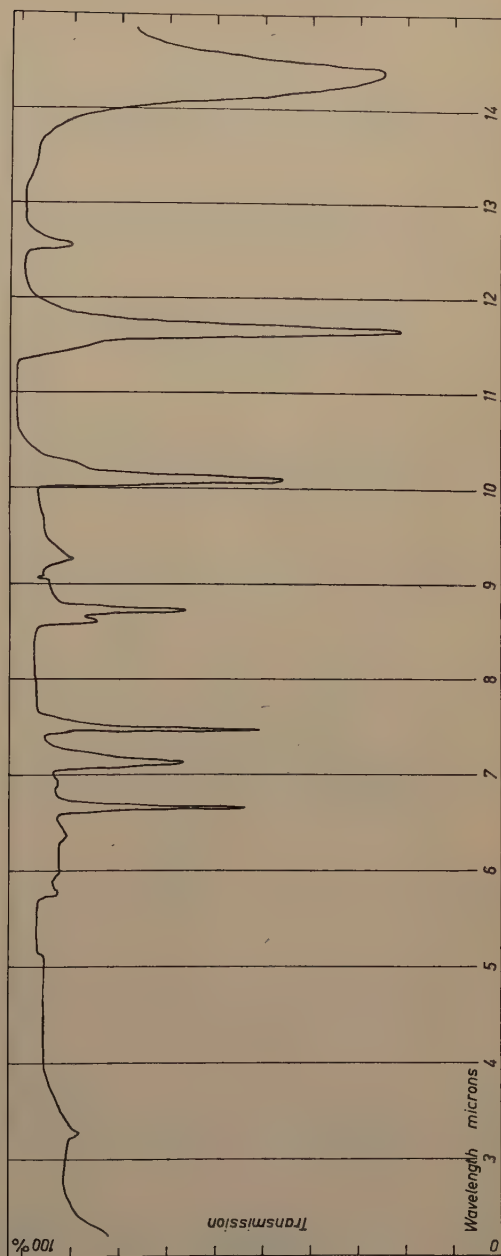


Fig. 3. The infrared spectrum of 2,3-dibromothiophene, prepared from 2-thiophenecarboxylic acid (liq.).

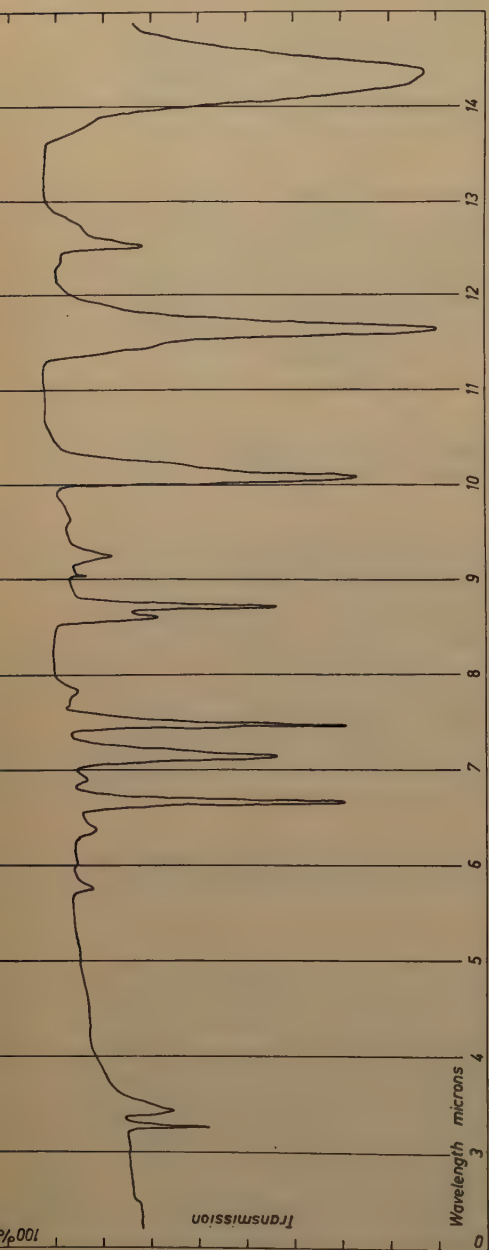


Fig. 4. The infrared spectrum of 2,3-dibromothiophene, prepared from 3-bromothiophene by bromination (liq.).

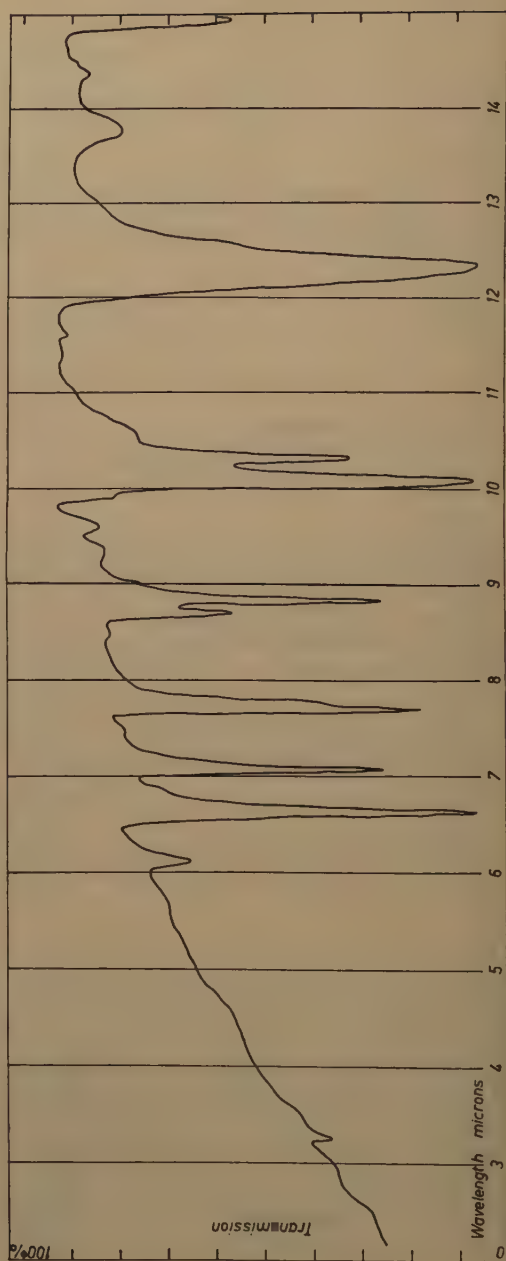


Fig. 5. The infrared spectrum of 2,3,5-tribromothiophene, prepared from 3-bromothiophene by bromination (in pressed KBr).

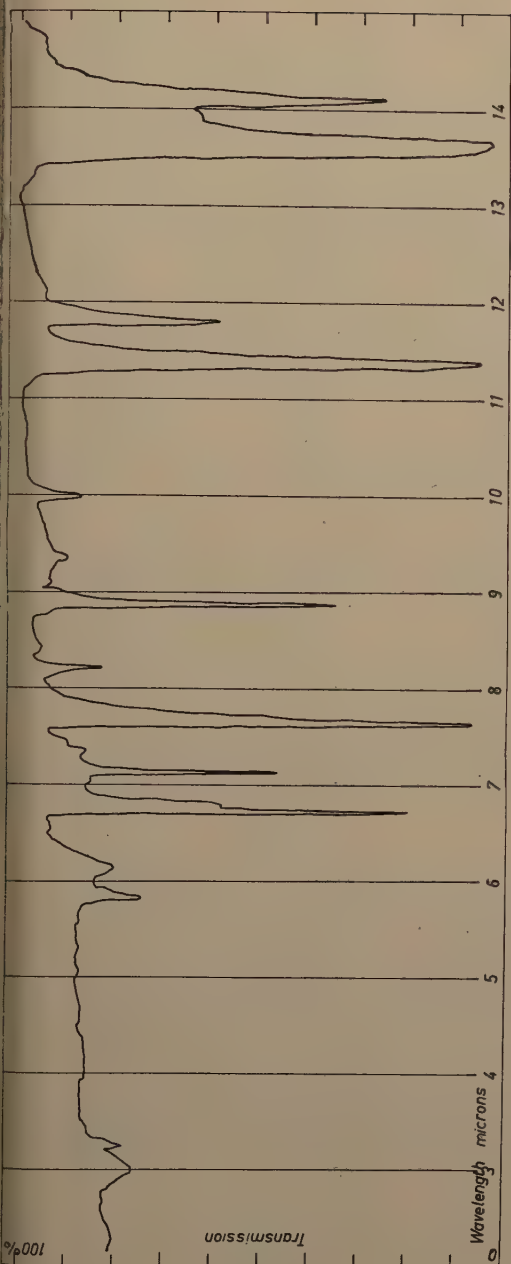


Fig. 6. The infrared spectrum of 2,3,4-tribromothiophene (in pressed KBr).

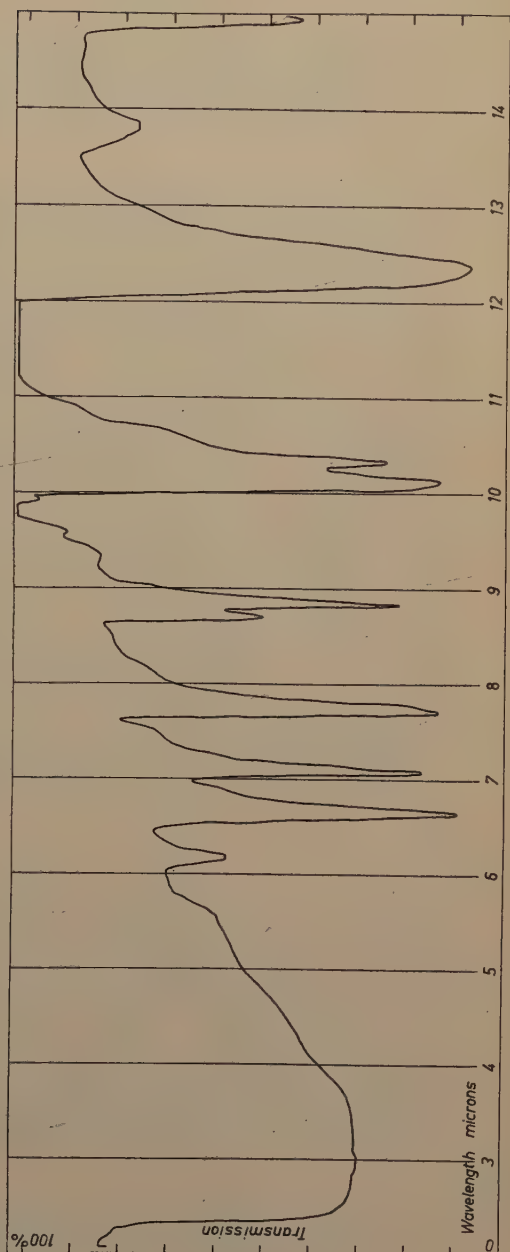


Fig. 7. The infrared spectrum of 2,3,5-tribromothiophene, prepared by bromination of 2,5-dibromothiophene (in pressed KBr).

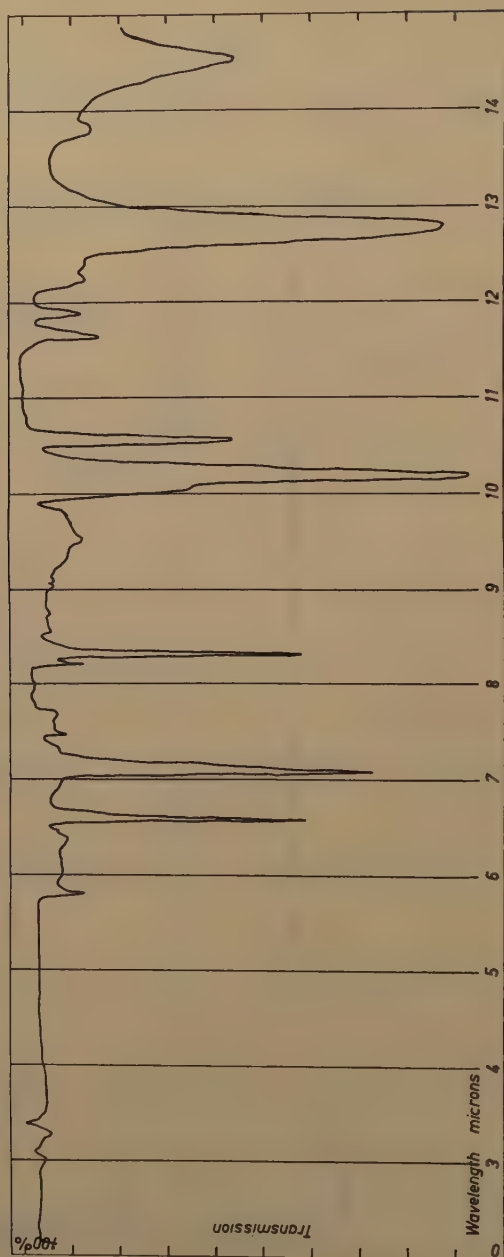


Fig. 8. The infrared spectrum of 2,5-dibromothiophene, prepared by the bromination of thiophene with bromine (liq.).

with light petroleum (b.p. 40–60°C) (50 ml) which precipitated traces of succinimide. After filtration and removal of solvent, the residue was distilled. Unchanged 2-bromothiophene (3 g) was followed by the main fraction, 2,5-dibromothiophene. B.p. 79–81°C/9 mm Hg. Weight 36.8 g. Yield 76 %. Infrared spectrum, Fig. 2.

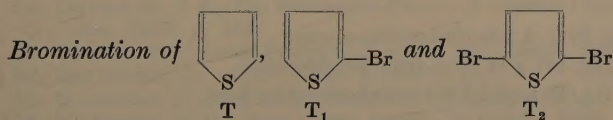
Bromination of 3-bromothiophene

(a) *With one mol. of bromine.*—To a solution of 16.3 g of 3-bromothiophene in 25 ml of dry benzene, cooled to 0°C by an ice-water bath, 16 g of bromine in 25 ml of dry benzene was added during 90 minutes with stirring. The solution was stirred for 8 hours, after which it was warmed under reflux for 15 minutes. No evolution of hydrogen bromide was observed. After the solution was poured into water, the organic layer was separated and the water phase shaken with ether. The combined extracts were shaken with 10 % sodium hydroxide solution and finally with water. After being dried over sodium sulphate and freed from solvent, the residue was distilled, giving 2,3-dibromothiophene as the main fraction. B.p. 89–91°C/13 mm Hg. Weight 18.4 g. Yield 76 %. The substance was identified by infrared analysis, Figs. 3 and 4.

(b) *With two mol. of bromine.*—To a solution of 16.3 g of 3-bromothiophene in 50 ml of glacial acetic acid, cooled to 0°C by an ice-water bath 32 g of bromine, dissolved in 50 ml of glacial acetic acid was added dropwise. The solution was stirred for 16 hours at room temperature and was then heated for 35 minutes at a temperature of 100°C. After most of the acetic acid had been removed under reduced pressure, the residue was dissolved in 50 ml of ether, which was then shaken free from acid first with 10 % sodium carbonate solution and then with water. The ether solution was dried over sodium sulphate, concentrated and distilled giving 2,3,5-tribromothiophene as the main fraction. B.p. 120–122°C/11 mm Hg. Weight 22.4 g. Yield 71 %. Recrystallisation from petroleum ether gave white needles with melting point 28–29°C. Infrared spectrum, Fig. 5.

Preparation of 2,3,4-tribromothiophene

To a solution of 34 g of 2,3,4,5-tetrabromothiophene in 100 ml of absolute ether, cooled to 0°C in an ice-water bath and in nitrogen atmosphere, 85 ml of 1 *M* butyllithium (in ether) was added during 10 minutes with stirring. After stirring for another 25 minutes the solution was poured into cold water. The organic layer was separated, the water phase shaken a couple of times with ether and after the combined extracts had been shaken with water they were dried over sodium sulphate. The solution was concentrated and then distilled giving 2,3,4-tribromothiophene as the main fraction. B.p. 132–134°C/11 mm Hg. Weight 23.1 g. Yield 85 %. Recrystallisation from petroleum ether gave long white needles with melting point 42–44°C. Infrared spectrum, Fig. 6.



In Table 1 are given the results of 8 brominations of thiophene compounds, here named T, T₁ and T₂. The usual procedure was used and the bromination of 2,5-

Table 1.

Bromination				Result (mol).			
Reactant	Mol bromine	Time (hours)	Temp. (°C)	2-bromo- thiophene	2,5-dibromo- thiophene	2,3,5-tri- bromo- thiophene	2,3,4,5-tetra- bromo- thiophene
	Mol reactant						
T	3:1	22	20		0.27 ^a	0.66	0.01
T	3:1 ^b	48	20	0.19	0.48	0.30	
T	1:1	6	20 (90) ^c	0.26	0.17	0.01	
T ₁	0.25:0.25	2	20	0.02	0.18	0.01	
T ₁	0.5 :0.25	10	0	0.04	0.14		
T ₂	0.69:0.63	15	50		0.08	0.49	0.01
T ₂	1:1	36	20		0.37	0.51	0.04
T ₂	0.66:0.33	15	20 (90) ^d			0.02	0.27

^a The dibromo fraction was shown to be the pure 2,5-isomer. Fig. 8.

^b The bromination was carried out in benzene. Heating with alcoholic NaOH for 24 hours.

^c The temperature was 90°C during the last three hours of the bromination. Heating with alcoholic NaOH for 12 hours.

^d During the last five hours of the bromination the temperature was 90°C.

dibromothiophene may be taken as a typical example. As the boiling points of the four different dibromothiophenes (32) are very similar it is impossible to separate them by distillation with the apparatus available here. Instead infrared spectra have been used for identification, thus providing a high degree of certainty.

Bromination of 2,5-dibromothiophene

153 g of 2,5-dibromothiophene was placed in a flask equipped with a mechanical stirrer, a separatory funnel and a double surface reflux condenser carrying an outlet tube connected to a gas trap. The bromine (110 g) was run in at such a rate that little, if any, of it was carried over with the hydrogen bromide. This required about 90 minutes. After that the flask was heated for 15 hours (50°C) and then for one hour at 100°C. This last treatment drives the bromination to completion and removes impurities which gradually evolve hydrogen bromide. The solution was then poured into water, the organic phase separated and washed first with diluted sodium hydroxide and then with water. Finally it was dried over sodium sulphate. From the first water phase some organic products were recovered by shaking it with ether a.s.o. Distillation gave

Fraction I. B.p. 79–81/9 mm Hg, 20 g of 2,5-dibromothiophene.

II. B.p. 118–120/9 mm Hg, 157 g of 2,3,5-tribromothiophene.

III. B.p. 159–163/9 mm Hg, 11 g of 2,3,4,5-tetrabromothiophene.

Fraction II was identified as 2,3,5-tribromothiophene by its infrared spectrum, Fig. 7.

SUMMARY

The products formed by the reaction of bromine with thiophene have been investigated. Only the substitution products 2-bromo, 2,5-dibromo, 2,3,5-tribromo and 2,3,4,5-tetrabromothiophenes have been identified and no addition compounds could be isolated. It is proposed that the mechanisms of chlorination and bromination of thiophene differ in certain respects.

3-Bromothiophene is now easily available by an improved method of preparation.

The mono bromination of 3-bromothiophene has been shown to give 2,3-dibromothiophene exclusively.

By bromination of 2-bromothiophene with N-bromosuccinimide 2,5-dibromothiophene has been produced.

The preparation of 2,3,4-tribromothiophene has been performed by dehalogenation of tetrabromothiophene with butyllithium.

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REFERENCES

1. MEYER, V., *Ber.* 16 (1883), 1465.
2. TÖHL, A., and SCHULTZ, K., *ibid.* 27 (1894), 2834.
3. KRAUSE, E., and RENWANZ, G., *ibid.* 62 (1929), 1710.
4. MOZINGO, R., HARRIS, S. A., WOLF, D. E., HOFFHINE, C. E., EASTON, N. R., and FOLKERS, K., *J. Am. Chem. Soc.* 67 (1945), 2092.
5. BUU-HOI, NG., *Ph. Ann.* 556 (1944), 1.
6. DJERASSI, C., *Chem. Rev.* 43 (1948), 271.
7. STEINKOPF, W., and OTTO, A., *Ann.* 424 (1919), 61.
8. STEINKOPF, W., *ibid.* 430 (1922), 78.
9. HURD, C. D., and ANDERSON, H. J., *J. Am. Chem. Soc.* 75 (1953), 3517.
10. ROSENBERG, J., *Ber.* 18 (1885), 1773.
11. MEYER, V., and KREIS, H., *ibid.* 16 (1883), 2172.
12. VOLHARD, J., and ERDMANN, H., *ibid.* 18 (1885), 454.
13. COONRADT, H. L., HARTOUGH, H. D., and JOHNSON, G. C., *J. Am. Chem. Soc.* 70 (1948), 2564.
14. COONRADT, H. L., and HARTOUGH, H. D., *ibid.* 70 (1948) 1158.
15. STEINKOPF, W., *Die Chemie des Thiophenes*. Theodor Steinkopf, Dresden, 1941, p. 41.
16. BLICKE, F. F., and BURCKHALTER, J. H., *J. Am. Chem. Soc.* 64 (1942), 477.
17. DEWAR, M. J. S., *J. Chem. Soc.* 1946, 406, 777.
18. DEWAR, M. J. S., *The Electronic Theory of Organic Chemistry*. Oxford University Press, London, 1949, p. 168.
19. BROWN, H. C., and BRADLY, J. D., *J. Am. Chem. Soc.* 74 (1952), 3570.
20. MELANDER, L., *Arkiv Kemi* 2 (1950), 211.
21. HAMMOND, G. S., *J. Am. Chem. Soc.* 77 (1955), 334.
22. LAWESSON, S.-O., *Acta Chem. Scand.* 10 (1956), 1020.
23. DE HEER, J., *J. Am. Chem. Soc.* 76 (1954), 4802.
24. MELANDER, L., *Arkiv Kemi* 8 (1955), 361.
25. LAWESSON, S.-O., *ibid.* 11 (1957), 325.

S.-O. LAWESSON, *Thiophene Chemistry. Part VI*

26. LAWESSON, S.-O., *ibid.* 11 (1957), 317.
27. STEINKOPF, W., and KÖHLER, W., *Ann.* 532 (1937), 250.
28. HALVARSON, K., and MELANDER, L., *Arkiv Kemi* 8 (1955), 29.
29. INGOLD, C. K., *Structure and Mechanism in Organic Chemistry*. Cornell University Press, Ithaca, N.Y., 1953.
30. HARTOUGH, H. D., *Thiophene and Its Derivatives*. Interscience Publishers, Inc., New York, N.Y., 1952.
31. CHAPMAN, N. B., and WILLIAMS, J. F. A., *J. Chem. Soc.* 1952, 5044.
32. STEINKOPF, W., *Ann.* 513 (1934), 281.

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